ANATOMY OF THE VEGETATIVE ORGANS OF THE PINEAPPLE, ANANAS COMOSUS (L.) MERR. (*Continued*)[16]

II. THE LEAF[17, 18]

BEATRICE H. KRAUSS

The leaf: Initiation and early development

The leaves of the pineapple originate acropetally as lateral projections of the apical growing region of the stem axis (figs. 21–23, 32). The initiating cells arise close to the apex of the stem from cells which are several cell generations removed from the initials in the main axis, as is the case in most plants (33). Both the surface layer of the meristem, the dermatogen, and the deeper, subdermatogen layers of cells are involved (fig. 23).

The somewhat limited scope of these investigations permitted neither a detailed study of the exact layers of apical stem cells involved in the initiation of the leaf primordium nor a determination of the specificity of the initial cell layers of the young leaf with respect to the tissues ultimately derived from them, such as several workers have done for various plants in recent years (18, 27, 28, 43, 44, 60).

Initiation of a leaf primordium is first evidenced by the apparently more rapid division of cells in both the surface layer and several—usually two or three—layers beneath this, in a region delimited as described below. Continued division and growth of the cells in all these layers produce a perceptible swelling. SCHUEPP (124*a*), after demonstrating that the intensity of cell division is approximately the same in all portions of the growing point of a stem, maintained that adjustment to the continued increase in volume is brought about by folding of the outer cell layers at definite points. These surface folds represent the leaf primordia.

In the pineapple the swelling appears as a crescent-shaped, rounded, collar-like ridge, the base of which extends over a lateral distance equal to approximately two-thirds of the stem circumference in this region (fig. 15). Since rapid increase in the diameter of the growing region of the stem axis continues, the base of the leaf primordium is pushed away from the center of the apex. At the same time there is a rapid extension of the primordium from the surface of the stem apex. Since the rate of growth, at first, is greater in the abaxial portion (to become the lower side of the leaf), the primordium curves adaxially so that, for a time, it arches over the apical growing region and the successively formed primordia (figs. 21–23, 32).

The apical portion of the primordium and subsequently of the young leaf is a growing region which remains more meristematic than the remainder of the organ. Through activity in this apical region the primordium—and, later, the

[16] The first section of this paper appeared in Vol. 110, No. 2, of the BOTANICAL GAZETTE. Footnote and figure numbers are continued in sequence.

[17] A concluding section of this paper will appear in a succeeding issue of the BOTANICAL GAZETTE and will include "Literature Cited."

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young leaf—increases in length for a time, and some of the fundamental structure of the leaf is laid down (see below). Apical growth appears to cease early; further growth and differentiation are a result of the activity of an intercalary meristem at the base of the leaf (figs. 29, 33) and of general division and extension throughout the organ.

A median procambial strand is early differentiated by periclinal divisions in the cells of the central region. Through continued differentiation of procambial cells from the ground meristem the strand develops both acropetally toward the primordium tip and basipetally until it joins the procambial tissue formed in the apical meristem of the stem (figs. 22, 24). Some authors (**28, 44, 60**) have adopted GRÉGOIRE's and LOUIS' ideas (see these authors) on the formation of procambial cells in connection with the development of "foliar buttresses" (*soubassements foliaires*). These are described as lateral portions formed through a transverse expansion of the stem apex at the points of primordia initiation. The formation of these structures precedes the actual emergence of the foliar primordium, and the first primordial procambial tissue is found in such a "buttress." There is no evidence of the formation of such buttresses in the pineapple stem. ENGARD (**36**) contended that the "foliar basements," as he called them, do not exist as morphological units, since the development of the primordium is a continuous process, and that all degrees of development of the foliar protuberance before it assumes the earliest recognizable differentiation of parts are adequately covered by the familiar term *primordium*.

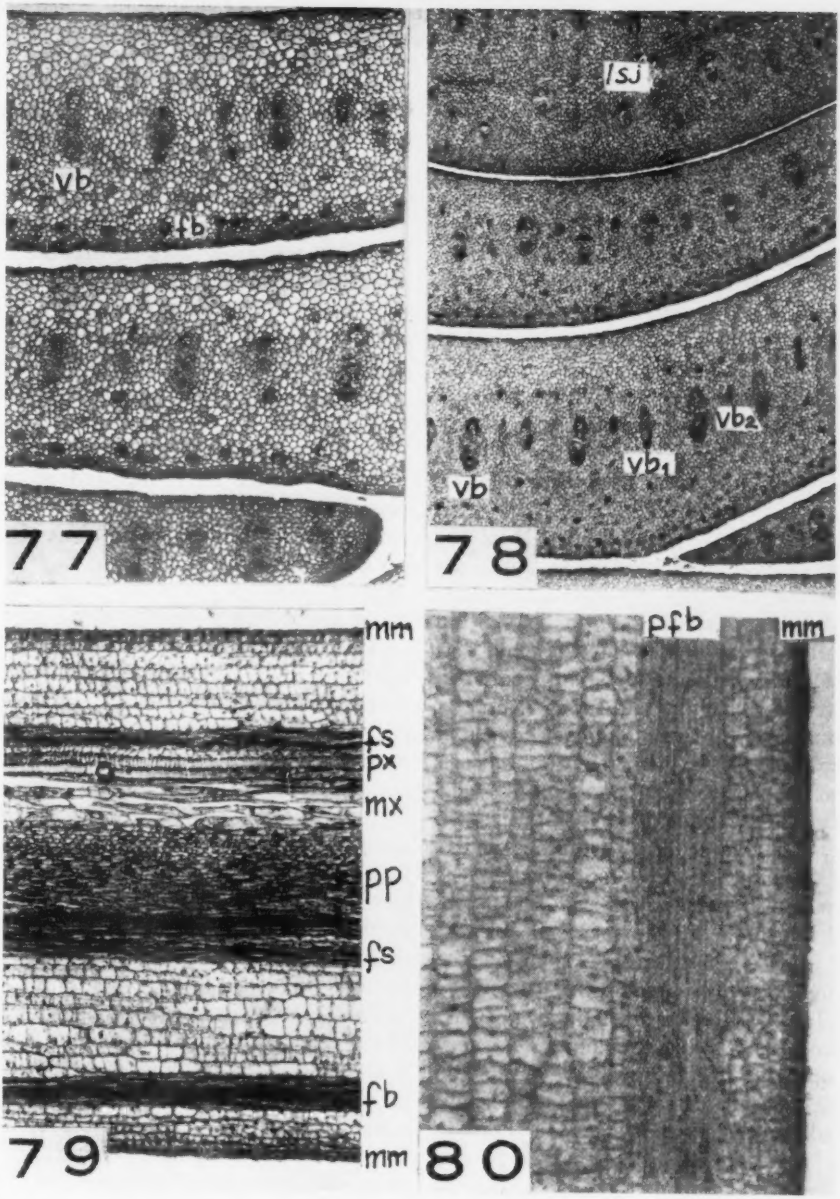
The epidermis is also early differentiated from the dermatogen and is continuous with the epidermis of the stem (fig. 23).

Subsequent to the early stage of the leaf—the primordium just described—it rapidly elongates, with a tapering toward the tip also characteristic of the fully developed leaf. It seems probable that the tapering is the result of two processes: a more rapid median apical growth during the period in which the rate of growth is also greater in the abaxial portion of the leaf primordium and young leaf and, later, a cessation of meristematic activity at the leaf margins, first at the tip and progressively toward the base. A distinct marginal meristem does not exist, but the marginal regions of the young leaf consist of cells which remain meristematic longer than the differentiating remainder (fig. 80).

With elongation there is further differentiation of cells to form additional procambial strands, lateral to the first-formed median one (figs. 13–15, 25).

Further development in the young leaf brings a change in its ground tissue; the meristematic appearance disappears, as is especially evidenced in the vacuolation of the cells, and this tissue now consists of cells which, in shape, resemble the unspecialized mesophyll cells in the chlorophyllous portions of older leaves. The cells are subglobose with small intercellular spaces, more prominent in transverse sections. At this stage all cells of the ground tissue—all cells exclusive of those in the epidermis and procambial strands—are of this type.

In subsequent development within this ground tissue the cells toward the upper surface of the leaf become larger and more elongated in the direction perpendicular to the leaf surface than those nearer the lower surface. On the lower side of the leaf the isodiametric cells have smaller diameters, and the intercellular



FIGS. 77–80.—Early development of leaf. Figs. 77–78, transverse sections of basal portions of developing leaves to show differentiation of later-formed vascular bundles, vb_1 and vb_2, between first-formed bundles, *vb;* and isolated fiber strands, *fb*. Fig. 78, leaves at later stage of development than in fig. 77. *lsj*, leaf-stem "junction." Figs. 79–80, vertical longitudinal sections of younger portion of leaves to show differentiating vascular bundle, fig. 79, and meristematic margin (*mm*) and prosclerenchyma cells (*pfb*) of fiber strand, (fig. 80). *fs*, fibrous sheath; *px*, protoxylem; *mx*, differentiating metaxylem; *pp*, differentiating metaphloem; *fb*, fiber strand.

spaces are more prominent in transverse sections. Also, in the interim, more procambial strands have formed, and cells have been added to and differentiated within the first-formed strands. The procambial and vascular strands lie approximately halfway between the upper and lower epidermis, in a zone extending from one margin to the other (figs. 13, 14). Also, strands made up of thin-walled cells elongated in the direction of the leaf axis and with small diameters are to be found on the lower side of the leaf, between the zone of procambial and vascular strands and the lower epidermis. These are the cells from which bundles of sclerenchyma cells—the isolated fiber strands which extend the length of the leaf parallel to the vascular bundles—are differentiated (figs. 77–80). At this stage these presclerenchyma cells have thin walls of cellulose. About the time that the metaxylem and metaphloem are differentiating in the first-formed vascular bundles, strands of similar presclerenchyma cells develop above and below each of the vascular bundles on the metaxylem and metaphloem sides (figs. 85, 90). These later form the fibrous "caps" or "sheaths" so well developed in the pineapple leaf. The walls of these presclerenchyma cells do not begin to thicken until all the vascular elements of the bundle are fully developed.

This description of the internal structure of the young developing leaf very much resembles that of the basal, i.e., older, leaves of a 3- to 4-month-old seedling described by MILES THOMAS and HOLMES (**98**) except that the former —the young leaf of the older plant—is without chlorophyll and may thus be called a prechlorophyllous leaf. The structural description presented here applies to the five or six youngest leaves of the rosette, which are inclosed and shaded by the immediately surrounding longer and partially chlorophyllous leaves. The longest of these prechlorophyllous leaves may be as much as 5 or 6 cm. in length.

Later development of the young elongating prechlorophyllous leaf includes the formation of additional procambial strands between the existing procambial and vascular bundles, further differentiation of all vascular and sclerenchyma tissue, and the development of scalelike trichomes on both the upper and the lower epidermis and of stomata on the lower surface only. The development of these two latter structures and of the silica bodies which begin to form in the epidermal cells at about the same time are described later.

The fully developed leaf

A leaf of the type briefly described in Part I, page 167, and represented by the first leaf in the upper group in figure 7 is portrayed here. Such a leaf is fully developed but not wholly mature, from the standpoint of its anatomy, and is, physiologically, very active. It is fully developed in the sense that all tissues and cells are formed, but not mature in that some cell walls become thicker and that further lignification, suberization, and cutinization of elements may be expected.

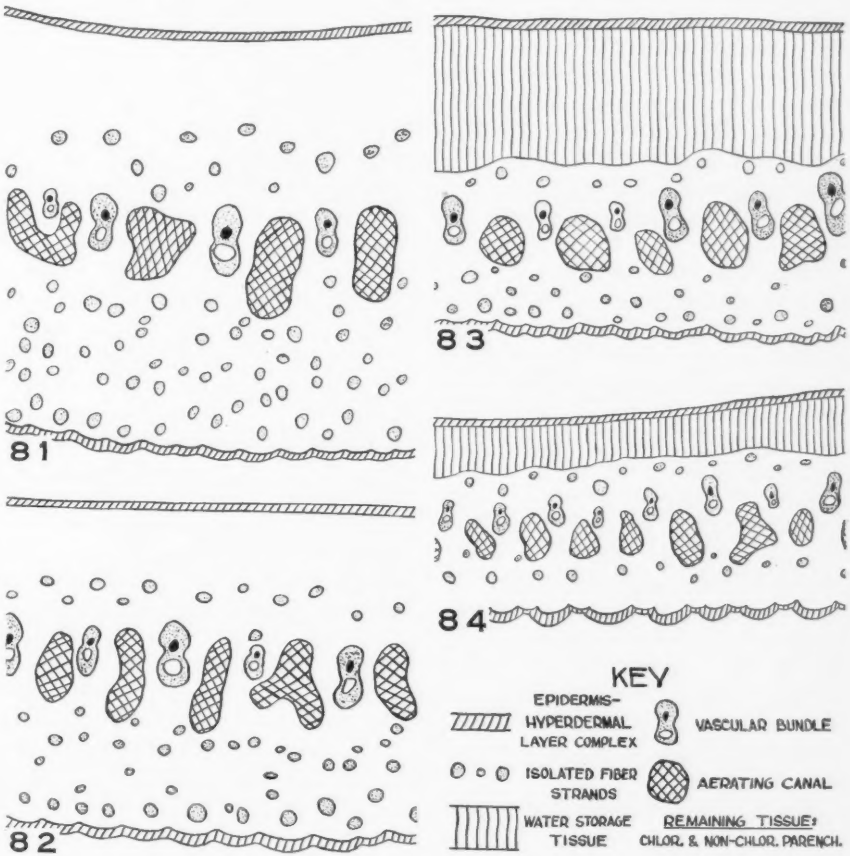
This leaf consists of the main portion of the blade or lamina—the chlorophyllous part—and an expanded, nonchlorophyllous base. The blade is lanceolate in form and about 90 cm. long, 6.5–8 cm. broad, and 2–3 mm. thick (when turgid) at its widest portion; the base is 4–6 cm. long, about 12 cm. wide where it is "attached" to the stem, and 4 mm. thick. SOLEREDER and MEYER (**141**) wrote of bromeliaceous leaves as generally differentiated into a sheath and a blade or

lamina. Other authors, however, referred to a "sheathing broad base" (**167**), or a "basal sheathing portion" (**94**); this latter interpretation, of the pineapple leaf, at least, seems the more correct to this writer.

The transition zone between the blade proper and the base, including that narrower portion called the "neck"—because of the slight narrowing of the width in this portion—is subchlorophyllous. This transition region is 4–6 cm. long, about 5.5 cm. broad, and 4 mm. thick in the median portion.

The thickness of the leaf gradually decreases toward the margins and tip.

The superficially dissimilar regions noted above are characterized by differences in internal structure as well, although previous writers have but briefly (**94, 110, 120, 126, 141**), if at all, remarked upon this fact in their descrip-



FIGS. 81–84.—Diagrammatic representation of transverse sections of leaves cut midway between margins to show proportion of different tissue types in different leaf regions. Fig. 81, basal—nonchlorophyllous. Fig. 82, transition—subchlorophyllous. Figs. 83–84, blade proper—chlorophyllous: middle of blade, fig. 83, and toward tip, fig. 84. ×15.

tions of leaves of the pineapple, or of any bromeliaceous plant, for that matter. MEZ (94) noted differences, essentially quantitative (in volume of different tissues), between these two leaf regions but stated that these dissimilarities were not always present and were not important (from a systematic point of view). Other authors (110, 120, 141) maintained that differences in structure between basal and apical portions of leaves of the rosette type of Bromeliaceae have physiological significance: the basal portion plays the role of roots by virtue of the absorbing hairs here, while the upper portion serves the functions of the usual foliage leaf (120), or the function of assimilation is depressed in the basal tissue as a result of reduced light and small quantities of (or no) chlorophyll here (110).

Throughout its length, except at its tip, the leaf is channeled, i.e., concave, on the upper side so that in transverse section it is crescent-shaped (fig. 6*A*). This type of structure, along with other features, makes for the rigidity characteristic of pineapple leaves, despite their great length. SCHWENDENER (127) first indicated that the resistance to bending (*Biegungsfestigkeit*) of leaves is correlated with the shape of their cross section. Later, RICHTER (110), in his study of the leaves of various bromeliaceous plants, including *A. comosus*, demonstrated, empirically, that leaves with a crescent-shaped cross section offer fifty-one times more resistance to bending than leaves with flat blades having the same surface area and thickness.

The upper surface of the leaf is quite smooth, while the lower surface is minutely furrowed in the direction of the leaf axis. This characteristic of the pineapple leaf has been mentioned before (13, 22, 65) and is found in representatives of a number of other genera of the Bromeliaceae (94). In transverse sections of the younger portion of the leaf the ridges appear in the form of a great number of tiny juxtaposed arches which MEZ (94) compared to "corrugated sheet iron" structure (*Wellblechconstruction*). As seen in transverse sections of younger basal portions of the leaf the slopes of the ridges are moderate and the width of the furrows narrow; in older portions of the leaf the furrows are deeper and wider at the bottom, and the slopes of the ridges steeper (figs. 81–84).

The function of the intercalary meristem—the basal meristematic region of the leaf—in common with the apical and marginal meristems, is to add length to this organ and to lay down additional fundamental structures. Since the apical and marginal meristems persist but for a comparatively short period, the greater part of the increase in leaf size (aside from that attributable to the division and extension of the individual cells) and of the formation of the permanent tissue is due to the activity of this intercalary meristem. The basal growth of pineapple leaves which results is well demonstrated, externally, in those basal leaves of the plant which have constrictions (fig. 6*B*). As noted previously (Part I, p. 165) these constrictions mark the division between the more mature acroscopic portion of the leaf, formed during the development of the propagating material—slip, shoot, or crown—on the mother plant before setting out in the ground, and the younger basiscopic portion formed when the plant resumed growth after planting.

When the intercalary meristem has ceased to function and all the tissues of the leaf have become differentiated or permanent, the basal portion of the leaf remains in what may be regarded as an arrested state of development. In other

words, at maturity this basal nonchlorophyllous region resembles, in many respects, the undeveloped, immature stages of the now acroscopic regions.

Internally, the leaf is differentiated into several clearly defined types of tissues. These consist of a parenchymatous ground tissue which is further differentiated, in the chlorophyllous region, into a water-storage tissue, on the upper, and the mesophyll, on the lower sides (figs. 83, 84). In the basal nonchlorophyllous region there is no such difference, all parenchymatous ground tissue being essentially alike (figs. 81, 82). Conspicuous even to the unaided eye in transverse or paradermal longitudinal sections are the alternating vascular bundles and central aerating canals, the latter a specialized type of mesophyll. In transverse sections the cross-sectional areas of alternately spaced vascular and aerating tissue extend in a row from one margin of the leaf to the other, approximately midway between the upper and lower epidermis (figs. 81–84). In median paradermal longitudinal sections the bundles and canals are seen to traverse the length of the leaf, parallel to one another until they converge toward the tip. The venation is thus of the type commonly known as parallel, or "longitudinally striated" (30).

There are approximately one hundred bundles and an almost equal number of central aerating canals in a fully developed leaf such as described.

Also traversing the length of the leaf are numerous isolated fiber strands, located chiefly on the lower side (figs. 81–84). Enveloping the leaf is the epidermis which, except for portions of the stomatal and trichome structures, forms an unbroken, protective layer over the entire surface and is continuous with the epidermis of the stem. Immediately beneath the single layer of epidermal cells is a layer usually called the hypodermis by most authors, but called the hypodermal layer in this paper to avoid the objections of certain investigators; it consists of one to several layers of sclerenchyma and collenchyma-like cells; this also forms an unbroken covering for the internal tissues of the leaf, except in those portions interrupted by parts of the stomatal and trichome structures.

Longitudinal vascular bundles

In the fully developed leaf the longitudinal vascular bundles are of two general sizes, i.e., with different cross-sectional areas, the smaller being spaced alternately between the larger and in line with the latter, or slightly above them (figs. 82–84). Earlier authors (**13, 30, 110, 167**) have described or illustrated the presence of large and small bundles, the two sizes alternating with one another, in bromeliaceous plants in general, and in many other monocotyledons. Although this type of arrangement predominates, there is a rather frequent variation of two or even three smaller bundles placed between two large bundles (fig. 83). In development the smaller bundles are differentiated and mature later (figs. 77, 78).

In cross section the longitudinal bundles are approximately oblong or oval in outline (figs. 78, 85, 90, 91); often the larger bundles are greatly elongated in the vertical direction (fig. 86). The average size of the larger bundles, in cross section, is about 1.0 × 0.4 mm. and that of the alternating smaller ones, about 0.6 × 0.3 mm.

The vascular bundles diminish in cross-sectional diameter as the margins and tip of the leaf are approached, as do the amounts of the various vascular tissues in each bundle. As the leaf narrows

toward the tip, two or more individual bundles converge and form aggregate bundles which consist of the elements of the formerly individual bundles. These aggregate bundles diminish in size and quantity of elements, converging nearer the tip to form new aggregates. At the tip the remaining bundles form into one strand (fig. 97). Aside from the differences mentioned later, the vascular bundles vary little in structure or size in the nonchlorophyllous, the subchlorophyllous, and the chlorophyllous portions of the leaf.

XYLEM AND PHLOEM.—The bundles are of the collateral type characteristic of many monocotyledonous plants and are similar in many features but not identical with those described for the stem. The phloem is on the lower side and the xylem on the upper side, since these two tissues maintain their relative positions as the leaf traces pass from the stem into the leaf.

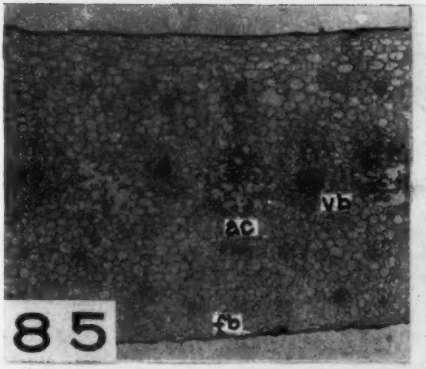
In development the procambial strands appear very early in the promeristematic tissue of the leaf primordium. Within these strands differentiation proceeds rapidly (figs. 77–79), the order of differentiation and maturation proceeding as in the vascular strands in the stem. The protoxylem is the first part to become differentiated; the first elements formed are tracheids with annular secondary wall thickenings, with spiral tracheids appearing later. Differentiation of the metaxylem, which consists of porous vessels with scalariform and reticulate pitted walls, follows very closely that of the protoxylem. In the fully developed leaf the xylem portion of the bundle consists of protoxylem, in the form of the remnants of the ruptured annular and spiral tracheids and the resulting rather large protoxylem lacunae; metaxylem, consisting of several porous vessels with scalariform and reticulate pitted walls arranged irregularly, as seen in transverse sections of bundles; and the accompanying parenchyma (figs. 90–93).

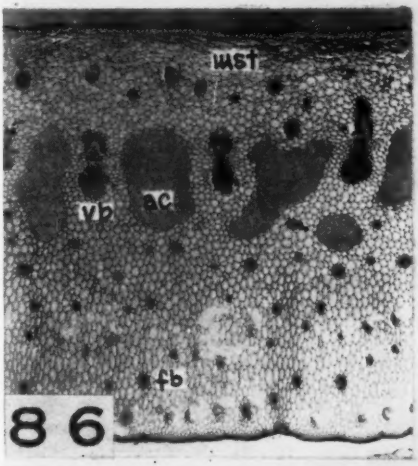
The protophloem is differentiated soon after the protoxylem; its cells differ little from the procambial cells from which they are derived. The metaphloem is formed after the metaxylem and consists of sieve tubes and companion cells. In the fully developed leaf the phloem portion of the bundle in cross section consists wholly of metaphloem in the form of a circular or oval group of sieve tubes and companion cells and accompanying parenchyma, since the cells of the protophloem are early crushed, although their remnants may be found (figs. 90–92, 94).

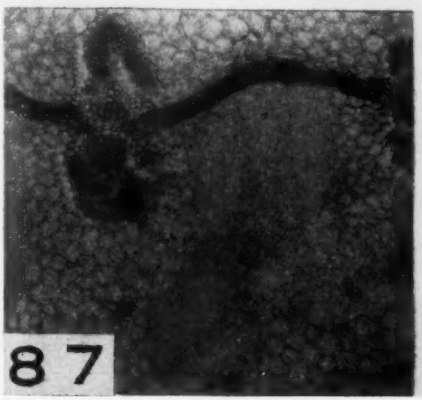
The metaxylem and metaphloem are formed from the procambial portion of the strand which remains between the protoxylem and protophloem. In the mature bundle the xylem and phloem portions are separated by parenchyma and the cells of an endodermis-like sheath.

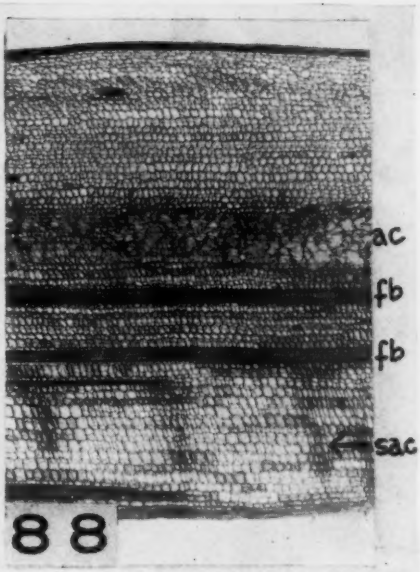
Surrounding the vascular elements of the mature bundles are three distinct bundle sheaths. The outermost is the parenchymatous sheath. Adjacent and internal to it is the fibrous sheath or, more correctly, subsheath, since it does not completely surround the bundle. Within the fibrous sheath and adjacent to the vascular elements is an endodermis-like sheath.

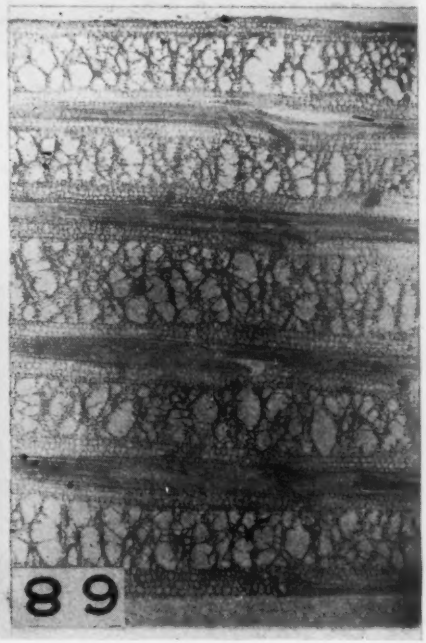
FIBROUS SHEATH.—The large, crescent-shaped caps of fibers are the most characteristic feature of the bundles in the mature leaf. These strands of sclerenchyma cells are found adjacent to both xylem and phloem (fig. 91) in contrast to the location of the fibrous subsheath adjacent to the phloem only in the bundle in the stem (figs. 64, 65). By reason of these fibrous structures the vascular bundles in both leaf and stem of the pine-



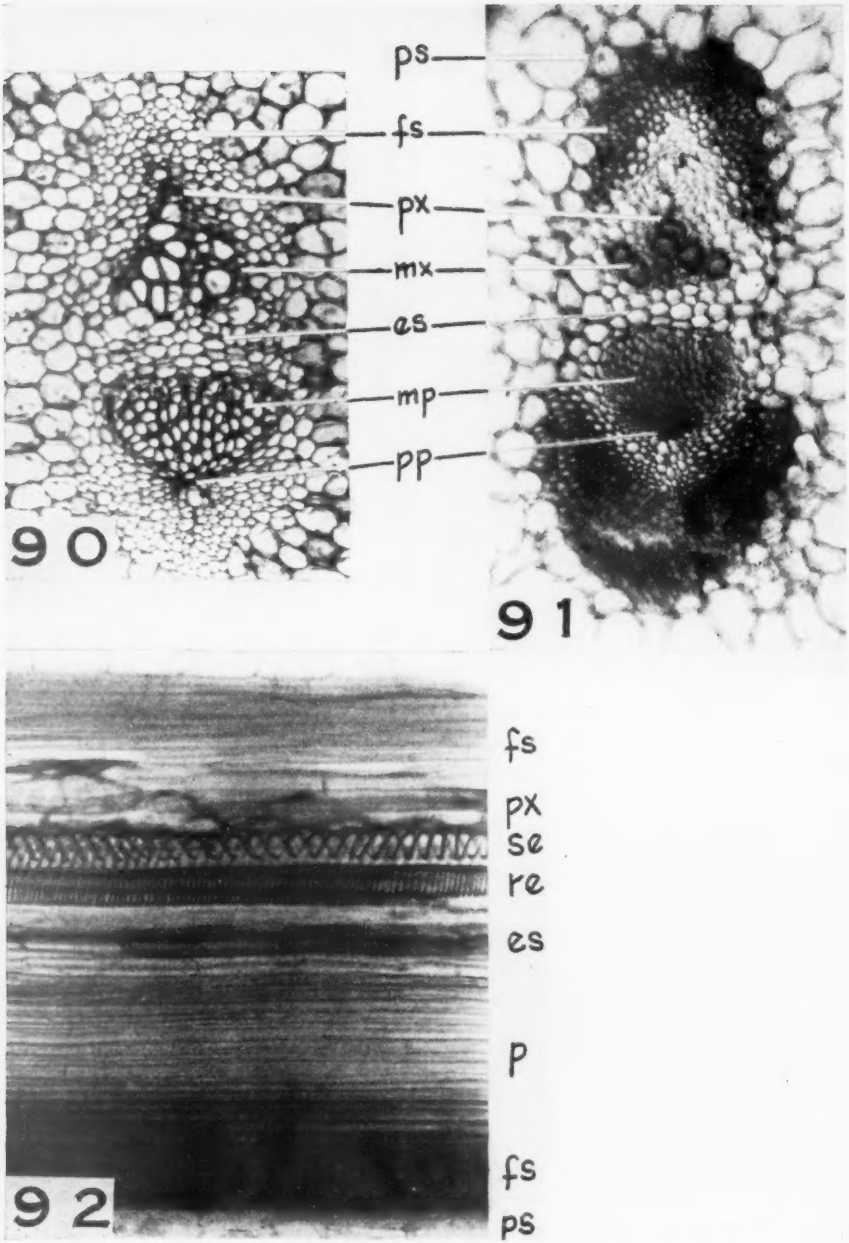




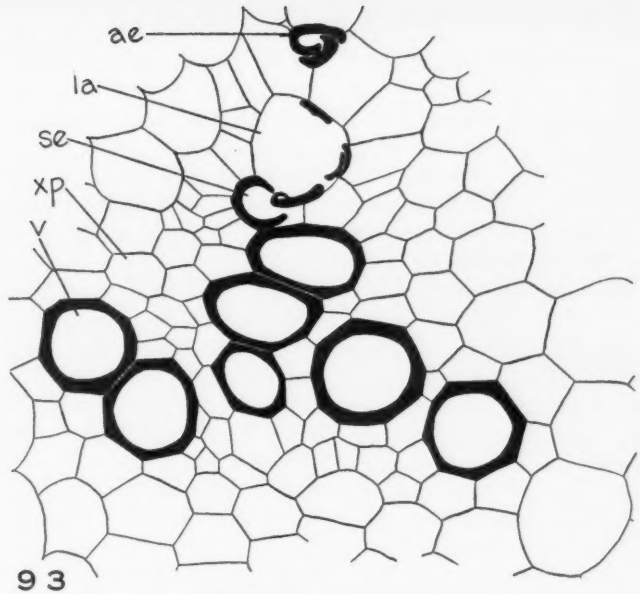




FIGS. 85–89.—Aerating canals. Figs. 85–87, transverse sections: of younger (basal) portion of leaf, fig. 85, to show differentiating tissue of central aerating canal, vascular bundle, and isolated fiber strand; of mature leaf in chlorophyllous region, fig. 86, with central aerating canal, vascular bundles, isolated fiber strands and collapsed water-storage tissue; of portion of mature leaf, fig. 87, to show transverse branches (in longitudinal section) passing from one longitudinal vascular bundle to another (in cross section) at edge of central aerating canal. Fig. 88, vertical longitudinal section of basal portion of leaf with central aerating canal, isolated fiber strands, and secondary aerating canals indicated by denser appearance of cells at *sac*. Fig. 89, paradermal longitudinal section of mature leaf to show alternating stripes of central aerating canals and mesophyll tissue with longitudinal vascular bundles (only fibrous sheaths were cut). *ac*, central aerating canal; *fb*, isolated fiber strand; *vb*, longitudinal vascular bundle; *wst*, water-storage tissue.



FIGS. 90–92.—Longitudinal vascular bundles in leaf, in transverse, figs. 90–91, and vertical longitudinal, fig. 92, sections. Figs. 90, 92, immature and fig. 91, mature bundles. *ps*, parenchymatous sheath cells (with tannin in fig. 91); *fs*, cells of fibrous sheath; *px*, protoxylem; *mx*, metaxylem; *se* and *re*, spiral elements, with steep and flat spirals, respectively; *es*, cells of endodermis-like sheath; *mp*, metaphloem; *pp*, protophloem; *p*, phloem. See figs. 93–94 for details of bundle tissues.



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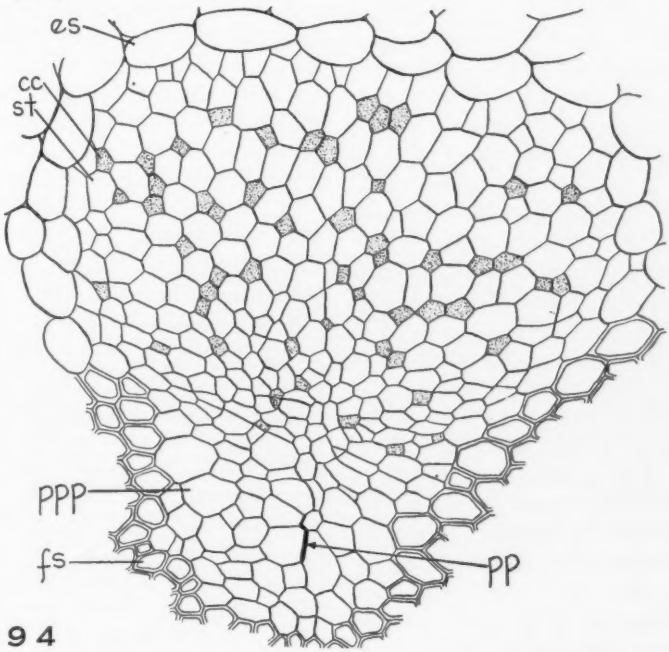

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FIGS. 93–94.—Detail of xylem (fig. 93) and of phloem (fig. 94) portions of longitudinal vascular bundles. *ae* and *se*, annular and spiral elements of protoxylem, respectively, and lacuna (*la*) formed by pulling-away of parenchyma from elements; *xp*, xylem parenchyma; *v*, vessels; *es*, endodermis-like sheath cells; *cc*, companion cells; *st*, sieve tube; *ppp*, phloem parenchyma; *fs*, fibrous sheath cells; *pp*, protophloem. ×300.

apple may properly be called "fibrovascular bundles," a term which has, in general, been superseded in descriptions of plants in general by the more correct (in most cases) "vascular bundle." As EAMES and MACDANIELS (33) have pointed out, however, this fibrous sheath adjacent to the conducting strand is morphologically not a part of the vascular tissue.

Because of its two-sided reinforcement with mechanical tissue, this type of bundle has appropriately been called an I-form beam (girder) bundle. This descriptive terminology of this characteristic cross-sectional shape of bromeliaceous leaf bundles has been adopted by various authors (98, 126, 141). RICHTER (110) found this bundle type in all Bromeliaceae he studied, placing *A. comosus* in his group II*c*—those Bromeliaceae with simple I-form bundles in the center of the chlorophyllous parenchyma with fiber strands on the sides of pressure and pull. SCHULZ (126) also commented on the fact that this bundle structure contributed not only to the torsional strength of the leaf but to its elasticity.

The strands of mechanical tissue which form these caps on the upper and lower sides of the vascular bundles consist of sclerenchyma cells, or fibers, of great length. Previous authors have commented upon their great length in many Bromeliaceae (22); BERGMAN and WELLER (13) reported lengths of 450–650 μ for the sclerenchyma cells of the fibrous sheaths of vascular bundles in the stem (cells which resemble those in the leaf) in *A. comosus*. The present writer measured fibers even longer—ranging from 2500 to 5500 μ (2.5–5.5 mm.) in length. The ends of the sclerenchyma cells taper gradually to a point. The overlapping of the ends of the fibers and the interlocking of adjacent cells contribute much toward the mechanical strength of this tissue.[19]

The fibers in the fully developed leaf are sclerenchyma cells without a protoplast and with greatly thickened, lignified walls which finally become sclerosed with maturity. The walls are often so thick that only a very narrow lumen remains. They show no evidence of pits (even with magnifications of 800×), although pits were reported by BERGMAN and WELLER (13). The sclerenchyma cells in the leaf often, but not consistently, have septa (fig. 102). These dividing plates, which occur at intervals, are thin and of cellulose, in strong contrast with the heavy lignified or suberized lateral walls. That they are septa is shown by the lack of secondary walls corresponding to those on the lateral walls and by the fact that the middle-lamella-like septum does not extend to the middle lamella of the side walls but ends at the surface of the secondary wall. These septate sclerenchyma cells thus resemble the septate wood fibers associated with xylem (33).

In cross section the fibers are polygonal, with four to seven sides (fig. 94), and are usually wider in one direction than in the other.

THE ENDODERMIS-LIKE SHEATH.—The sheath which lies inside the fibrous sheath or subsheath and separates and surrounds the xylem and phloem portions of the vascular bundles of Bromeliaceae was first recognized and described by SCHWENDENER (127). He and later RICHTER (110), who studied this structure in greater detail in many Bromeliaceae (including *A. comosus*), and HARTWICH (58) called it an "endodermis." These authors believed that this bundle

[19] These fibers, in one variety of *A. comosus*, are used commercially to make a fine grade of fabric in the Philippine Islands known locally as "piña" cloth.

sheath resembled the endodermis in other organs of other plants in form and functions. RICHTER reported the radial walls of the cells as showing Casparian strips, and both he and SCHWENDENER credited the sheath with a mechanical function—strengthening the leaf and protecting the more delicate tissues within, especially the phloem—and a physiological function—providing an almost impermeable wall between the conducting and the surrounding tissues. A similar layer, called the endodermal sheath, is found surrounding the vascular bundles in ferns and grass leaves (**3, 30, 33, 127**), but EAMES and MACDANIELS (**33**) doubted whether the sheath in grass leaves is an endodermis in the strictly morphological sense. Since, however, the sheath in the pineapple leaf is comparable to the characteristic endodermal sheath in grass leaves and functionally probably serves somewhat the same purposes ascribed to an endodermis, it will be called an "endodermis-like sheath" in this paper.

Until once recognized, the cells of this sheath cannot always be very clearly distinguished from certain adjacent cells; upon examination of immature leaves or superficial inspection of mature ones, especially of transverse sections, the cells of the endodermis-like sheath might well be mistaken, as some authors have, for part of the xylem and phloem parenchyma (**65**) or for part of the fibrous sheath (**13, 64**). Some authors (**72, 94, 141**) recognized a differentiation of the "sclerenchyma sheath" into an outer portion made up of usual fibers and an inner portion composed of shorter cells with larger lumen and pitted walls. Although they did not name the two portions of their sclerenchyma sheath, these authors were without doubt describing the fibrous and endodermis-like sheaths, respectively.

The endodermis-like sheath was first definitely observed by this writer in mature parts of early-formed leaves—in leaves of seedlings and propagating materials. Once these cells were distinguished, however, they were easily recognized in all leaf bundles except the smallest, in which they may be absent. The cells resemble in most respects those described by RICHTER (**110**) for Bromeliaceae in general. They are isodiametric, elliptical or polygonal in cross section, and elongated in the direction of the leaf axis (figs. 92, 95). Although the end walls are usually transverse, or nearly so, they are not uncommonly oblique (fig. 95*A*). The walls of these cells are thicker than those of adjacent parenchyma cells but much thinner than those of the sclerenchyma cells forming the fibrous sheath (fig. 91). They are lignified and pitted, the pits being simple, with the apertures tending to be elliptical in outline in face view. The longer axis of the pit aperture is usually oblique (fig. 95*B*). No indication of the presence of Casparian strips, such as reported by RICHTER (**110**) for Bromeliaceae, was found on the radial walls of the cells of this sheath in the pineapple leaf.

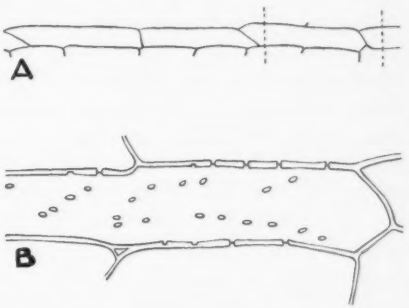


FIG. 95.—Endodermis-like sheath cells. *A*, longitudinal view of cells in endodermis-like sheath of longitudinal vascular bundle. *B*, detail of cell structure from portion delimited by broken lines in *A*.

SCHWENDENER (127) and RICHTER (110) both reported the presence of passage cells in this sheath, as it occurs in the Bromeliaceae; these are similar to the passage cells in the endodermis in other organs of other plants. RICHTER reported such passage cells in *A. comosus* to be adjacent to the xylem, directly against the vessels. The writer of this paper occasionally found such passage cells in the pineapple leaf but not necessarily limited to positions adjacent to the vessels.

The cells of the endodermis-like sheath mature late and until mature may be confused with either the bundle parenchyma or the fibrous sheath cells, as indicated previously. In the mature leaf the following features of the endodermis-like cells help to distinguish them from the others: they are longer than the parenchyma cells but considerably shorter than the fiber cells; although they are not much larger in cross section than the largest cells in the fibrous sheath, their walls are thinner and so their lumens are larger; their walls are pitted (these are seen to best advantage in longitudinal sections as in fig. 95) in contrast to the nonpitted walls of the parenchyma and sclerenchyma cells; and with proper stains, such as fuchsin and methylene blue (122*a*), their walls show a lignin reaction while those of the fibrous sheath show a suberin reaction.

PARENCHYMATOUS SHEATH.—Exterior to the fibrous sheath is the parenchymatous sheath of the Bromeliaceae (22, 156). The latter resembles the outer layer of specialized cells around the vascular bundles in grass leaves (3, 4, 33, 57, 60) —usually called the mestome sheath (33) or the parenchyma sheath (3).

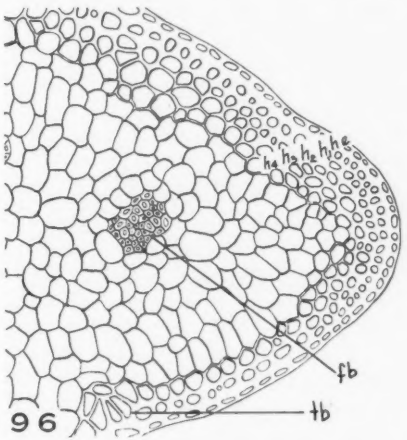
The parenchymatous sheath in the pineapple leaf, consisting of a single layer of cells noticeably smaller in cross section than those in the adjacent parenchyma, completely surrounds a bundle (fig. 91). This sheath is especially conspicuous in the subchlorophyllous region of the leaf. Here its cells are filled with chloroplasts, while the surrounding cells are without them. Both types of parenchyma have thin walls of cellulose. The sheath cells appear isodiametric in transverse sections of the leaf but are much elongated in the direction of the leaf axis. They lack chloroplasts only in the nonchlorophyllous basal region of the leaf, where, instead, they are often filled with starch grains as are the surrounding parenchyma cells.

Frequently but not consistently, the cells of the parenchymatous sheath contain a highly refractive, yellow or yellowish-brown substance, which has been shown to be tannin (156). It usually occurs in fresh, untreated leaves in the form of globules of varying sizes, although not infrequently the cells appear to be completely filled with a colored liquid.

In fresh sections of pineapple leaves the presence of the tannin in the cells of this sheath can be demonstrated by the classic ferric chloride test (improved by adding a little sodium carbonate [67*b*]), which gives a blue-green color to tannin or, better, by applying a strong solution of ferric acetate, which in combination with tannin forms a black precipitate (122*a*). In prepared sections the tannin is precipitated as a dark-red or reddish-brown granular material (fig. 91). JOHANSEN (67*b*) described this as phlobaphene, an oxidation product of tannin.

CEDERVALL (22) first reported this inclusion substance in the cells of the parenchymatous sheath of two of the many species of Bromeliaceae he examined; he described it as a "resin-like material" but wrote of no tests identifying the substance. The "oil drops" he described in the parenchymatous sheaths of other

species were probably also tannin, as WALLIN (156) pointed out. The "oil drops" reported in *Tillandsia dianthoidea* by BARBAINI (11) are likewise probably tannin. WALLIN was the first to identify the material correctly and described tannin to be characteristic for the Bromeliaceae in general, finding it in all the forty-three species (including *A. comosus*) he examined. Both CEDERVALL and WALLIN believed that this material served to prevent desiccation (of the vascular tissue) by reason of the osmotic pressure created by its presence.



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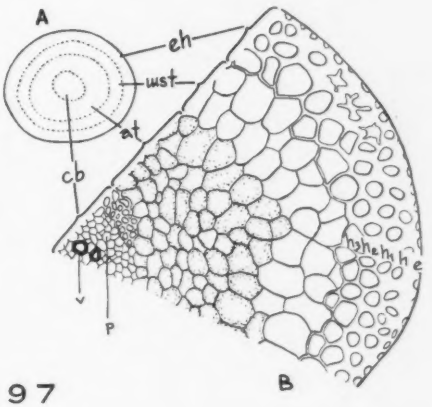

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FIGS. 96–97.—Transverse sections cut through margin, fig. 96, and tip, fig. 97, of mature leaf. Fig. 96, *e*, h–h_4, epidermal-hypodermal complex; *fb*, isolated fiber strand; *tb*, basal structure of scutiform trichome. Fig. 97, *A*, diagram to show relative amounts of various tissues and, *B*, sector of *A* to show details. *eh*, epidermis-hypodermal complex, consisting of one row of epidermal cells (*e*) and four rows (h–h_3) of hypodermal cells; *wst*, water-storage tissue; *at*, assimilatory tissue (mesophyll)—chloroplasts not shown; *cb*, consolidated or aggregate longitudinal central vascular bundle, consisting of vascular elements from several basiscopic bundles (*v*, vessel; *p*, phloem).

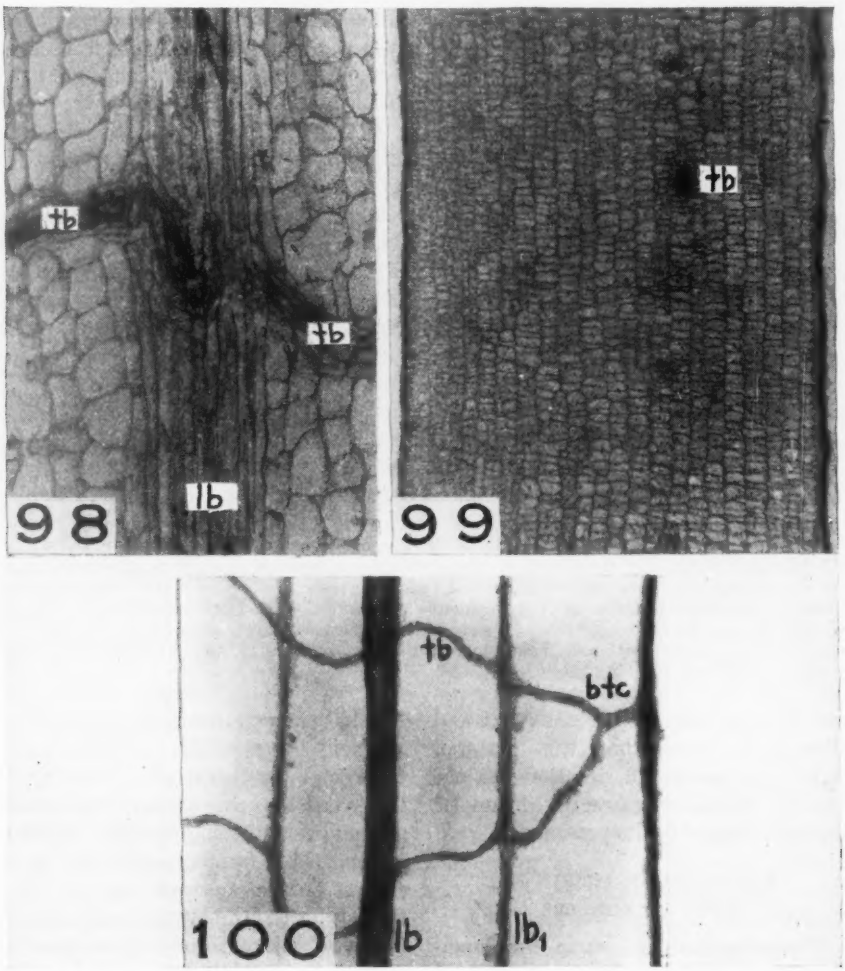
TRANSVERSE BRANCHES OF VASCULAR BUNDLES

Throughout their course the longitudinal vascular bundles are connected in a ladder-like manner by transverse branches (fig. 100). DEBARY stated (30) that this arrangement is found almost exclusively in the monocotyledons, and (30) has claimed that in those monocotyledonous leaves which have alternating large and small vascular bundles the transverse branches connect two bundles of equal size, passing over the intervening one, this is not true, except in rare instances, in the pineapple leaf; the usual thing is for the transverse branch to connect two adjacent longitudinal bundles. Sometimes one of the transverse branches itself divides, with the two latter branches connecting with the next longitudinal bundle (fig. 100).

The transverse branches usually pass

above or below the aerating canals, at the margin of the canal (fig. 87), although the canals are occasionally traversed by bands of parenchymatous tissue which serve as bridges through which passes such a transverse branch.

The cross-sectional area of the transverse branches is considerably less than that of the longitudinal bundles, chiefly because the former lack the thick, fibrous caps so characteristic of the latter. The vascular tissue of the transverse branch



FIGS. 98–100.—Transverse branches of longitudinal vascular bundles. Figs. 98, 99, differentiating vascular bundle and branch cells in immature region of leaf: fig. 98, paradermal and, fig. 99, vertical longitudinal sections. Fig. 100, portion of retted leaf, in surface view, to show alternating longitudinal bundles of larger diameter (*lb*) and those of smaller diameter (lb_1), connected in ladder-like manner by transverse branches (*tb*). Occasionally latter are branched, as at *btc*.

consists of one or more (usually not more than three) tracheids and/or vessels and a limited amount of phloem (fig. 101). The latter commonly consists of sieve tubes only (usually a maximum of ten), although companion cells may sometimes be present. The vascular elements are continuous with corresponding elements of the connected longitudinal bundles (fig. 98).

The xylem and phloem tissues are inclosed within two concentric sheaths, which resemble and are continuous with the endodermis-like and parenchymatous sheaths of the longitudinal bundles.

Functionally, the transverse branches serve a mechanical role, their lignified endodermis-like sheaths contributing to the protection of the leaf against lateral stresses, and a physiological role in that their vascular elements furnish a lateral conducting system to supplement the otherwise purely longitudinal system.

The transverse branches are formed early in the leaves at a time when the elements of the longitudinal bundles are differentiating (figs. 98, 99).

Isolated fiber strands[20]

In the description of the young prechlorophyllous leaf it was stated that isolated strands of thin-walled cells, with small diameters and elongated in the direction of the leaf axis, are formed in the lower portion of the leaf. In a transverse section of such a young leaf these strands appear in a row between the line of procambial and vascular strands and the lower epidermis (fig. 77). With further development within the leaf, additional strands of such presclerenchyma cells are formed (figs. 78, 85). The walls of these cells remain thin for some time but begin to thicken about the same time as the walls of the sclerenchyma cells which form the fibrous caps of the vascular bundles, i.e., after the vascular elements are fully developed. The sclerenchyma cells of the fiber strands resemble those of the fibrous sheaths.

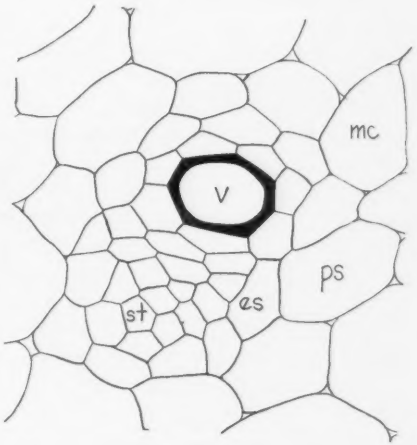


Fig. 101.—Transverse branch of longitudinal vascular bundle, in transverse section of leaf. Xylem consists of one vessel (*v*) only and surrounding parenchyma; phloem, of sieve tubes (*st*) only. Surrounding branch are cells of endodermis-like (*es*) and parenchymatous (*ps*) sheaths. *mc*, mesophyll cells.

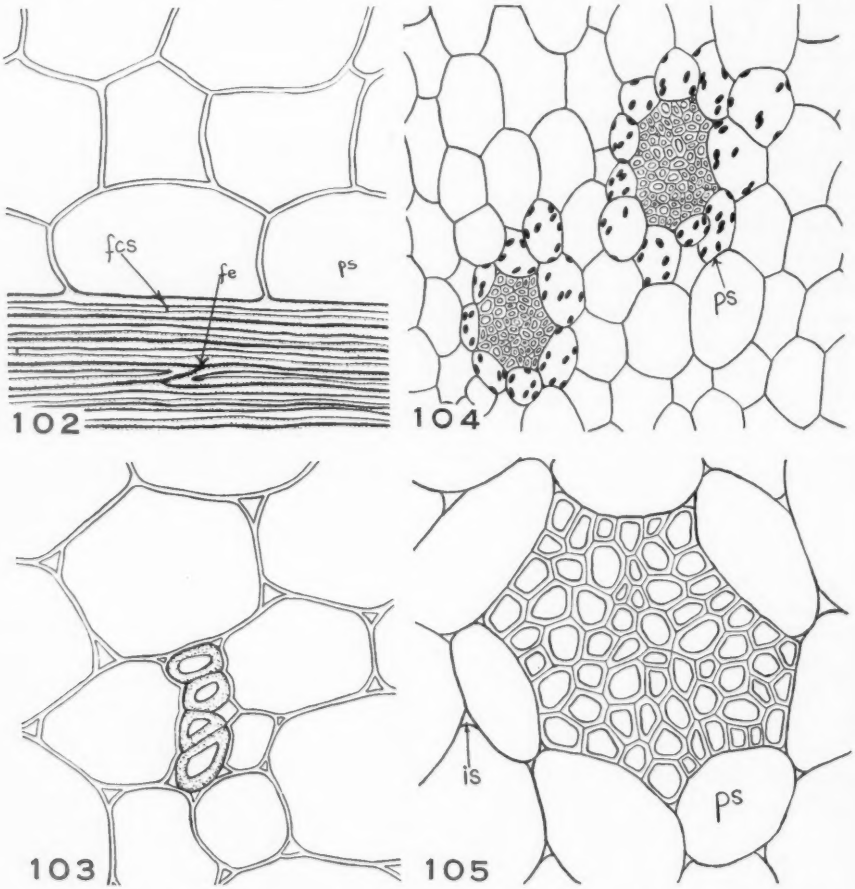
Mez (**94**) reported that such independent fiber strands were absent from a large number of genera of Bromeliaceae but included *Ananas* among the genera containing them. In the fully developed pineapple leaf these isolated strands of fibrous tissue, running parallel to the vascular bundles, are a conspicuous feature within the mesophyll, especially in that zone lying between the vascular bundles and the lower epidermis (fig. 86).

In transverse sections of leaves the diameter, number, and arrangement of these fiber strands vary with the region of the leaf. The individual strands con-

[20] The term "fiber strand" is used instead of the more common "fiber bundle" in order to avoid confusion with the term "fibrovascular bundle," at the suggestion of Dr. Eames (private communication).

tain from a single or a few (the less usual cases) to many sclerenchyma cells in cross section (figs. 103–105). The large strands are somewhat regularly arranged in rows, with scattered, smaller, strands between (figs. 83, 86).

In the nonchlorophyllous region of a fully developed leaf there are more fiber strands than in the other regions—usually three to six rows in the parenchyma below the row of vascular bundles and one to three above (fig. 81). SCHULZ (126) believed that the more equal distribution of fiber strands in the lower region of the leaf adds to the torsional strength of the leaf here. In the subchlorophyllous region the number of strands usually is less; there may be one



FIGS. 102–105.—Isolated fiber strands. Fig. 102, portion of vertical longitudinal section of mature leaf to show fiber ends (*fe*) and septum (*fcs*). Note thick walls of fibers. Figs. 103–105, transverse sections of strands in nonchlorophyllous (fig. 103), in subchlorophyllous (fig. 104), and in chlorophyllous (fig. 105) regions of fully developed leaf. Illustrations represent small, medium, and large strands, respectively—found throughout leaf. *ps*, parenchymatous sheath cells; *is*, intercellular space. Figs. 102, 103, 105, ×650; fig. 104, ×200.

or two less rows of fiber strands below and above the vascular bundles (fig. 82). In the chlorophyllous region there are usually one or two rows below the vascular bundles and one above (figs. 83, 84). They are more regularly arranged in this region than in the other two regions, with the strands in the two rows staggered (fig. 83). A large fiber strand in the lower of these two rows is usually opposite each ridge. Toward the tip of the leaf there is either only one row of fiber strands and that below the vascular bundles, or one row above and one below the vascular bundles (fig. 84). In all three regions the number and size of strands are less toward the margins.

At the very base of the leaf and at the tip these strands are reduced in diameter, with the numbers of fibers fewer and fewer as these regions are approached. The two ends of a strand may thus often consist of but a single sclerenchyma cell.

There are usually no fiber strands in the mesophyll between the vascular bundles and the alternating aerating canals, except in those cases where the vascular bundles are displaced from the row of aerating canals toward the upper side of the leaf. In this case one or two fiber strands may be found in this portion of the mesophyll (fig. 84). There are no fiber strands in the water-storage tissue in the subchlorophyllous and chlorophyllous regions or in the corresponding portion of the leaf, not differentiated into water-storage tissue, in the nonchlorophyllous region.

Other authors (**13, 22, 94, 126**) have described a varying number of rows of fiber strands in the pineapple or other species of *Ananas;* these are usually in far smaller number than noted in the present paper. The explanation for the difference probably lies in the sampling; greenhouse-grown plants have fewer fiber strands than field-grown plants and, as shown above, the numbers of strands vary from region to region. Since there are no fiber strands in seedling leaves, it is not strange that MILES THOMAS and HOLMES (**98**) did not mention them.

Each fiber strand is completely surrounded by a parenchymatous sheath, similar to if not always so distinct as the one described for the vascular bundles. This sheath consists of a single layer of cells which are usually noticeably smaller in cross section than the cells in the surrounding parenchyma. As in the case of those surrounding the vascular bundles, the sheaths inclosing the fiber strands are especially conspicuous in the subchlorophyllous region. Here the sheath cells contain chloroplasts, while they are absent in the cells of the surrounding parenchyma (fig. 104). In the nonchlorophyllous basal region the sheath cells, as well as the surrounding parenchyma, are usually filled with starch grains.

THE AERATING SYSTEM

The central aerating canals or channels are part of an extensive aerating system which extends through the leaf and furnishes a more or less continuous connection between the internal and external atmospheres. Secondary aerating canals connect the stomatal openings and substomatal aerating chambers at the surface of the leaf with the central aerating canals. The terms "central" and "secondary aerating canals" (*zentrale Atemkanale* and *sekundäre Atemkanale*) of LINSBAUER (**88**) are used in this paper because of their appropriateness.

CENTRAL AERATING CANALS.—The leaf contains a specialized type of spongy mesophyll which because of its structure and function may be called an "aerating tissue." This tissue is confined to isolated delimited zones and consists of an irregu-

lar, loosely arranged meshwork of stellate cells, with large intercellular spaces, the whole forming canal-like structures traversing the length of the leaf (figs. 88, 89). Because the aerating tissue is confined to these definite canal-like zones, the latter may well be called aerating canals or channels, adopting the terminology of HABERLANDT (**57**) for plants in general and of LINSBAUER (**88**) in the Bromeliaceae. Other terms which have been applied to this tissue in the pineapple or other members of the Bromeliaceae are air passages consisting of stellate bands or cells (**30, 126**); spongy parenchyma or tissue (**22, 110, 141**); air lacunae (**126, 167**); lacunar parenchyma (**30**); aeration chambers (**94**); aerating tissue (**13**); and aerenchyma (**13, 126**). The use of the last term is incorrect for this tissue, since the word is already used to designate a tissue of a different type formed by a phellogen layer and occurring in aquatic plants (**33**).

In a fully developed leaf the central aerating canals are aligned and alternate with the vascular bundles (fig. 89). This statement should be qualified somewhat by stating that, although in the basal region of the leaf the centers of the vascular bundles and aerating canals are approximately in the same plane (figs. 81, 82), in the older regions the bundles are displaced somewhat toward the upper epidermis (figs. 83, 84).

LINSBAUER (**88**), in studying various bromeliaceous plants, including *A. comosus*, found no indication of direct communication between the canals. Although in the greater number of cases there is no connection between canals, such communication does occur (see below). The canals pass through the length of the leaf uninterruptedly except for the occurrence of occasional bands of unspecialized mesophyll cells which serve as bridges for some of the transverse branches of vascular bundles.

In a transverse section of the leaf the canals vary in shape and diameter. They appear approximately circular or, more often, elliptical in outline. In many cases, however, especially in the chlorophyllous region, the cross-sectional outline is very irregular (figs. 81–86, 106). In some cases, instead of single canals alternating with vascular bundles, two such canals are continuously connected along their length by a bridge of similar aerating tissue, either above or/and below the intervening bundle, to form a horseshoe-shaped canal above or below the bundle (fig. 81) or a canal in the shape of a hollow cylinder surrounding the bundle. In vertical longitudinal and paradermal longitudinal sections of the leaf the canals appear as bands of the aerating tissue, with clearly defined boundaries, which are usually parallel in these views (figs. 88, 89).

The diameter in cross section of the central aerating canals is not uniform, even within any given leaf region. At the very base and at the tip of the leaf, the canals are smaller and the number of stellate cells is less than in the mid-region. The canals also are smaller in diameter at the margins of the leaf.

The stellate cells are arranged in a loose irregular meshwork throughout the canal; they do not, therefore, resemble the stellate cells arranged in diaphragms at intervals in the air passages which were described by DEBARY (**30**) as occurring in some plants. The individual stellate cell is irregular in shape, consisting of a central portion, roughly spherical, from which long tubular arms extend in all directions (figs. 106, 108). Although the number of such arms varies somewhat, the most usual number counted was seven. The radiating arms of one

cell connect with those of adjacent cells, a cross wall occurring where two arms meet (figs. 106, 108). In the nonchlorophyllous region of the leaf the cells of this aerating tissue appear to have arms of lesser diameter and greater length and to be arranged more regularly in rows parallel to the leaf surface than in the chlorophyllous portion just described.

The stellate cells have thin walls of cellulose and contain protoplasm, with some chloroplasts in the chlorophyllous region (fig. 108*C*) and starch grains in the nonchlorophyllous region. Occasionally a bundle of raphides of calcium oxalate is to be found in the central portion of the cell (fig. 108*B*).

The mesophyll cells bordering the canal represent a transition type between the cells of the aerating tissue and those of the surrounding mesophyll (fig. 107).

The aerating tissue is formed rather tardily in the developing leaf and is not present in the prechlorophyllous stage. MILES THOMAS and HOLMES (**98**) made no mention of an aerating tissue in the seedling and young plant. Examination of such leaves by the writer has shown that such tissue does exist in these very young leaves but that it is of a simple type and occurs in small amounts.

DEBARY (**30**), who noted the occurrence of similar tissue in many monocotyledons, including Bromeliaceae, described the formation of this tissue as follows: during the differentiation of this tissue, air spaces arise schizogenously—as cells within this area pass from the meristematic state, unequal growth in the surface of all cells causes processes to be put out at certain points; these processes grow into long arms, while at other points they are not formed. The ends of the processes of adjoining cells remain connected with one another; between the other parts of the cell surfaces the intercellular spaces are formed. According to whether the processes are short or extended as long arms and whether they are arranged in one or several radial planes, the forms of the cells and the intercellular spaces vary. Stages in the development of the aerating tissue are shown in figures 111–114.

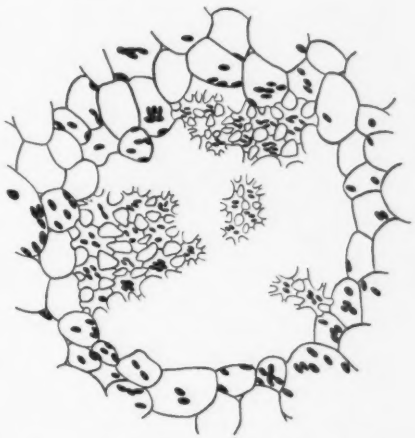


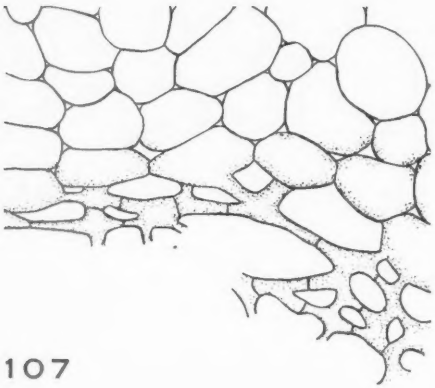
FIG. 106.—Cross section of cylindrical-shaped central aerating canal with some of stellate cells which constitute aerating tissue. ×150.

SECONDARY CANALS.—The secondary aerating canals follow a course—from the substomatal chambers through the mesophyll to the central aerating canals—which is almost perpendicular to the leaf surface. In the chlorophyllous and nonchlorophyllous regions these secondary canals are not always very clearly evident, but they can be located by the presence of the smaller mesophyll cells which border them. The presence of these smaller cells makes for a denser appearance (fig. 88). The secondary canals are very prominent in the subchlorophyllous region, especially in transverse sections, because these bordering cells contain chloroplasts, whereas the surrounding

cells (except for the parenchymatous sheaths around the vascular bundles and fiber strands) do not (fig. 109).

Whereas the central canals have a rather large diameter, the secondary canals have a small one, this being in most cases hardly more than the diameter of the bordering cells. The secondary canals are intersected at intervals by single cells which are attached to two opposing border cells with broad bases (fig. 110). Functionally, these intersecting cells evidently serve to support the walls of the secondary canals, i.e., they form a kind of truss which prevents the collapse of the bordering cells.

The air passages which form the secondary canals are schizogenous in origin.



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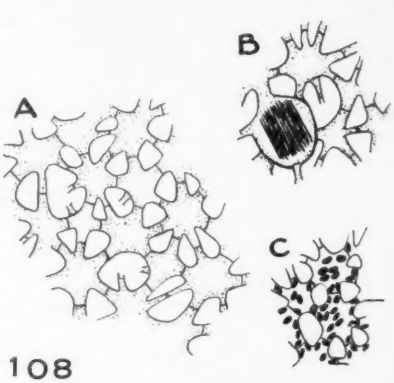

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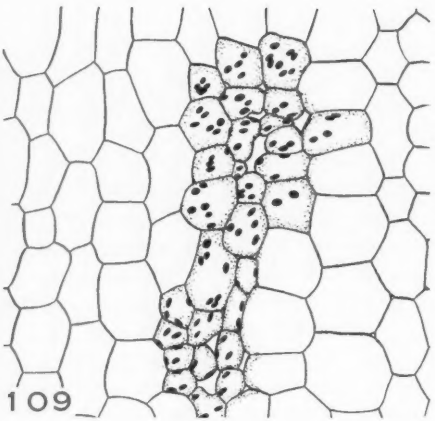

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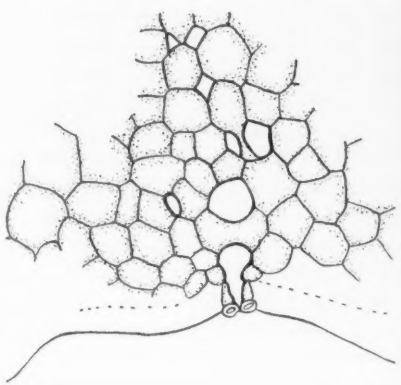

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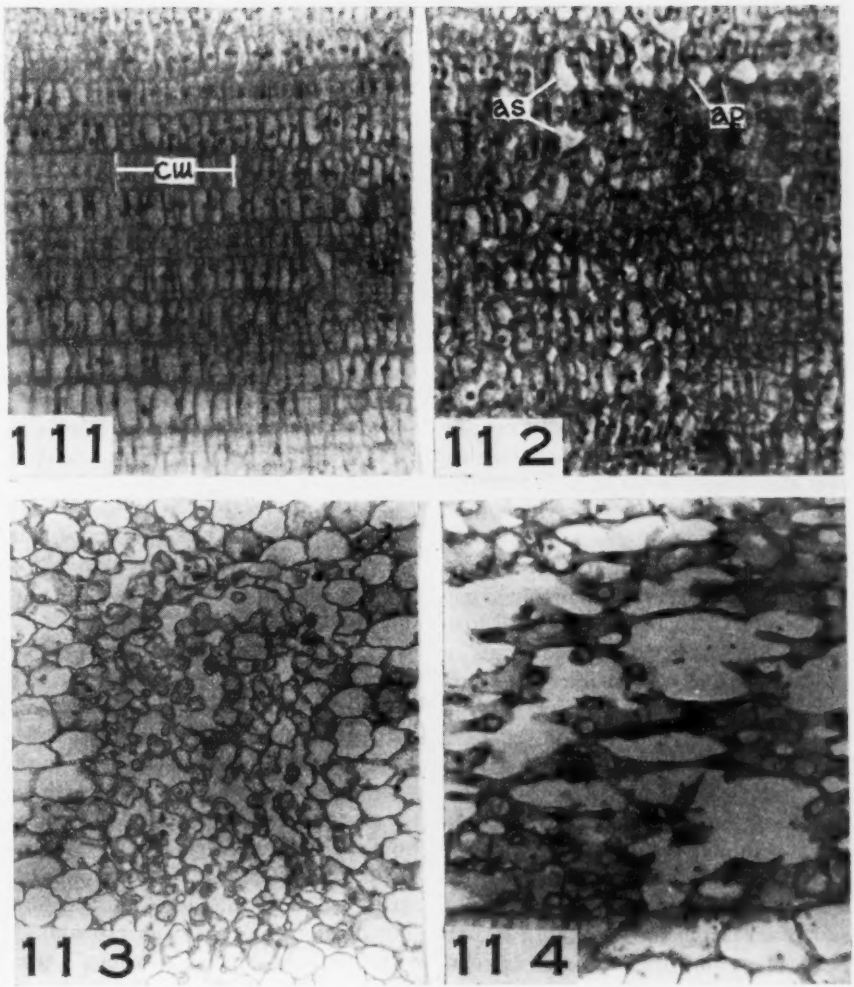
FIGS. 107–110.—Aerating tissue and canals. Fig. 107, transverse section of portion of basal, nonchlorophyllous region of fully developed leaf to show cells transitional between specialized stellate and unspecialized parenchyma cells bordering central aerating canal. Fig. 108, detail of stellate cells of aerating tissue: *A*, most usual type; *B*, calcium oxalate raphides in central portion; *C*, cells filled with chloroplasts. Fig. 109, paradermal longitudinal section of transitional, subchlorophyllous region of fully developed leaf to show several adjacent secondary aerating canals in cross section. Note chloroplasts in cells bordering canals and absence of these in remaining parenchyma. Fig. 110, transverse section of lower side of chlorophyllous region of fully developed leaf to show secondary aerating canal (in vertical longitudinal section) leading from substomatal chamber. ×200.



FIGS. 111–114.—Development of aerating tissue. Figs. 111, 112, 114, vertical longitudinal sections of young leaves from year-old plant. Fig. 111, linear rows of closely packed cells, with cross walls (parallel to leaf axis) beginning to form at *cw*, show first stage of differentiation. Fig. 112, regular alignment of rows is lost, divisions take place in several planes, and schizogenous intercellular spaces appear here and there (*as*) with armlike processes (*ap*) just beginning to form. Fig. 114, late stage of development; schizogenous intercellular spaces and armlike processes are conspicuous but characteristic form of stellate cells not yet evident. Fig. 113, transverse section of leaf to show same stage as depicted in fig. 114. ×200.

The intersecting cells probably represent a series of adjacent cells whose original common walls did not split.

FUNCTION.—Air chambers and passages occur both in plants which inhabit dry localities and in those which grow in water; they are chiefly associated with the latter plants. SCHIMPER (120) believed that the large air—intercellular—spaces occurring in the leaves of bromeliaceous plants represent an adaptation to the peculiar type of water absorption of the epiphytic Bromeliaceae, similar to that of swamp and aquatic plants. MEZ (94) disagreed with SCHIMPER's opinion, pointing out that such air spaces occur in the terrestrial forms of Bromeliaceae as well. MEZ regarded them as reservoirs, i.e., he believed that they provide an internal atmosphere for these plants. His opinion was that of the modern anatomist and physiologist—the oxygen given off in photosynthesis is evidently stored in these air spaces, to be used again in respiration; the carbon dioxide from respiration is then held here and used in photosynthesis (33).

LINSBAUER (88) agreed with MEZ's view and believed, further, that the aerating system in bromeliaceous leaves serves to inhibit transpiration, without affecting the assimilatory process, in the following manner. In an extensive system of air canals, as in these leaves, a loss of water vapor from the surrounding cells into the passages creates a pressure which would tend to depress further water vapor loss. However, since the maximum pressure lies deep within the leaf, with theoretically a potentially steeply descending gradient thus created between the central aerating canals and the surface of the leaf, and transpiration depending chiefly upon differences in water-vapor pressure between the internal and external air, loss of water vapor through the stomata might be potentially great were it not for the heavy covering of trichome heads over each stoma. On the other hand, since the entire system of air canals is bordered and interspersed with chlorophyll-filled cells so that the carbon dioxide is absorbed, a steeply descending gradient of carbon dioxide gas pressure between the leaf surface and the central aerating canals is created.

Thus the aerating system in the leaves of Bromeliaceae serves not only as a gas reservoir (33, 88) but also as a regulator of the entire gas exchange (88).[21]

WATER-STORAGE TISSUE

Nearly all investigators who described the leaf structure of bromeliaceous plants have commented on the water-storage tissue as one of the striking features of this family (22, 94, 110, 126).

It is because of the presence of this thick layer of water-storage tissue that many bromeliaceous plants, including the pineapple, are sometimes called "succulents." They are succulents in the sense that they are "juicy"—have "watery tissue." The presence of this water-storage tissue and certain other features justifies their being called xerophytes; according to EAMES and MACDANIELS' classification (33), they can probably be designated as "malacophyllous" xerophytes.

The terminology used for the water-storage tissue in this paper is the most generally accepted today (13, 57, 126, 141); other names very similar to the foregoing are "aqueous" or "water" tissue (22, 57, 72, 94, 104, 126, 167) or

[21] Experiments conducted by the present writer (77) showed that the average transpiration rate of pineapple plants is only 0.36 mg./sq. cm. of leaf surface.

water-storing tissue (**67, 98**). DEBARY (**30**) wrote of the tissue as "sap parenchyma," conscious of the cell contents but not mentioning the function of the tissue. Unfortunately, the diverse nomenclature and interpretation of this tissue by other authors has made for some confusion. These latter authors (**88, 110, 159**) have written of the water-storage tissue in terms of an epidermal or hypodermal structure, naming it an "epidermal" aqueous tissue or even a "hypodermis" (**110**).

In the pineapple leaf the water-storage tissue can be seen to best advantage in transverse or vertical longitudinal sections cut in the chlorophyllous region. Here there appears a sharp division of the ground parenchyma into distinct layers which are apparent even to the unaided eye (figs. 83, 84). The upper layer—the water-storage tissue—is colorless and translucent, while the lower portion—the mesophyll—is richly chlorophyllous. The proportional amount of leaf cross section occupied by each type of tissue varies with age, environment, and other conditions. In a turgid fully developed leaf from a field-grown plant under the conditions stated in the Introduction, in the general pineapple-growing district of Wahiawa, Oahu, the water-storage tissue occupies approximately half the leaf cross section in the median portion of the central region (fig. 83). Toward the tip and margins this tissue is lacking.

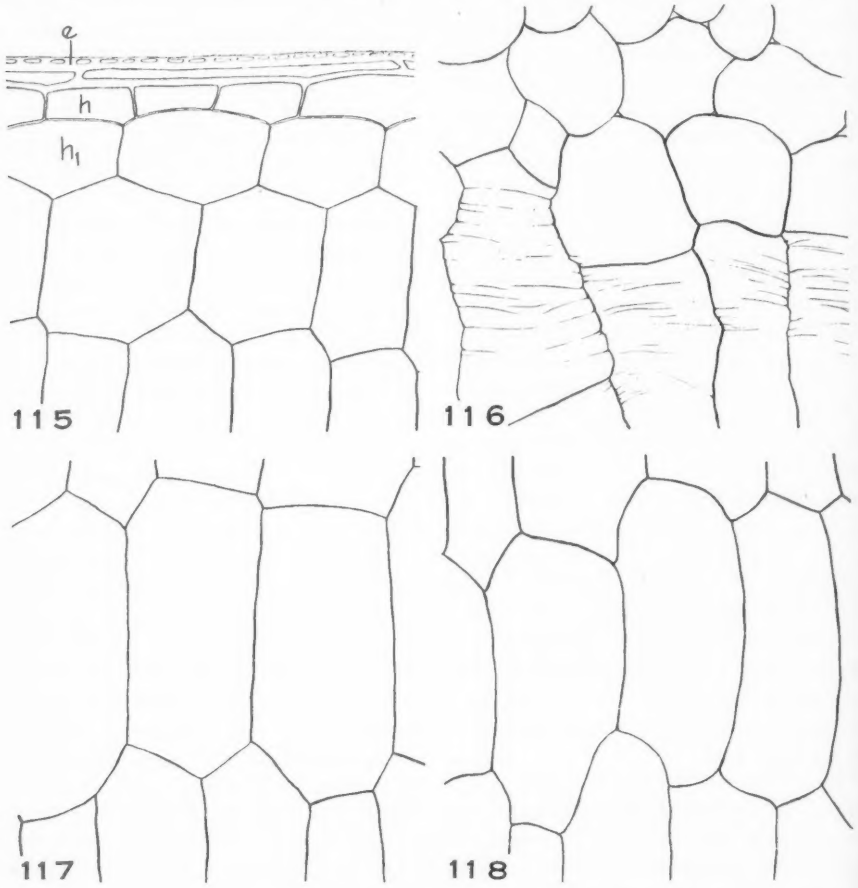
It is significant that the reports by other authors (**13, 22, 68, 84, 110, 141**) on the width of this tissue in the pineapple leaf show considerable diversity, presenting a range from one-fourth to two-thirds of the leaf cross section. If one assumes that the reports are all based on observations made in the same general region of turgid leaves of the same maturity,[22] there should be some explanation for the variations cited. Work by NIGHTINGALE (**102***a*) indicated that within the same district the thickness of the water-storage tissue varied with the growth status of the plants examined, including leaf "texture" as influenced by plant spacing (shading) and fertilization. More recent studies by the writer (**78**) showed that the proportion of water-storage tissue in the total thickness of the leaves from plants of the same age grown under practically identical cultural conditions, with water supply at an optimum, varied from one district to another where the altitude, total sunlight, temperature, and evaporating power of the air showed rather wide differences. This variation in the amount of water-storage tissue in leaves of plants of different growth types or grown in different localities undoubtedly has some physiological significance, but more study would be required before any definite statements could be made. The above observations of NIGHTINGALE and the writer were made incidentally in connection with experiments conducted for other purposes.

The water-storage tissue extends from the upper hypodermal layer to the mesophyll—the assimilatory or photosynthetic tissue. Although it is made up of several forms of cells, palisade-like cells predominate and occupy the greater volume of the layer. This form will be described later. The uppermost of the water-storage cells—the first two to six rows of cells beneath or adjacent to the hypodermis—are nearly isodiametric in cross section, i.e., in transverse leaf sections (figs. 115, 116), but elongated in the direction of the leaf axis. This cross-sectional diameter is greater in successively

[22] In young plants, it is true, the water-storage tissue may occupy only one-third of the leaf cross section.

deeper-lying cells (fig. 115), while the length in the direction of the leaf axis is less, so that in the deeper-lying layers these dimensions are nearly the same—the cells are spherical or more nearly cubiform (fig. 115).

The rows of cells beneath the first two to six rows are palisade-like. They are greatly elongated perpendicular to the leaf surface, this dimension being two to three times that of the paradermal diameter. RICHTER (110) described these perpendicularly elongated cells in *A. comosus* as "utriculiform"; it is true that in many cases these cells are "bladder-like" (fig. 118) rather than being the more usual "right parallelopipeds" (fig. 117), as BERGMAN and WELLER (13)



FIGS. 115–118.—Water-storage tissue. Figs. 115–116, vertical longitudinal and, figs. 117–118, transverse sections through upper side of central chlorophyllous region of fully developed leaf. Note increasing dimensions of transverse walls of successive water-storage cells beneath hypodermal layer, figs. 115–116; palisade-like (more usual form), figs. 115–117, and utriculiform, fig. 118, types of cells; and collapse of vertical walls, accordion-like, when water is withdrawn, fig. 116. *e*, epidermal cell; *h*, cell of first, and h_1, of second hypodermal rows. ×200.

designated the latter type. The palisade-like cells are usually arranged in definite, compact rows, so that there are no intercellular spaces.

The innermost row, or sometimes two rows, of cells of the water-storage tissue adjacent to the mesophyll also are palisade-like, but the cells are noticeably shorter in the vertical direction than those above them (fig. 119). The paradermal diameter is also less.

The cell walls in the water-storage tissue are thin and of cellulose; the cell contents appear colorless and consist of watery mucilaginous substances (**13, 30, 57**). Microscopic tests for mucilages made by the writer confirm this: the contents of cells of desiccated sections of this tissue were observed to swell with addition of water, and the watery, colorless contents of turgid cells formed into pale, yellowish-green globules on the addition of alcohol.

Functionally, this tissue is of great importance in the water economy of the plant. The various investigators who have described this tissue in bromeliaceous leaves, especially those with well-developed water-storage tissue, have emphasized this. During periods of ample water supply water is stored in this tissue, making the leaf turgid. In times of drought this water reserve is drawn upon to furnish water to other tissues, especially the adjacent mesophyll and more distant meristematic tissue. The mucilaginous substances in the cells of the water-storage tissue, because of their colloidal nature, serve not only to hold water or lose it very slowly under conditions where the external water supply is extremely low but to absorb water when this external supply again becomes available.

With loss of water from this tissue the thin, unsupported high walls of the palisade-like cells collapse undulously (see figs. 86 and 116 for walls wholly and partially collapsed, respectively). Upon the re-establishment of an external water supply and absorption of water into these cells, their walls become stretched once more as in the turgid leaf. This reversible process may evidently, if not carried too far, be repeated indefinitely in the life of a plant or be practiced experimentally without apparent injury.[23]

WESTERMAIER (**159**) was the first to describe this collapse of these cell walls with the withdrawal of water in Bromeliaceae; PFITZER (**104**) had previously described the cells in a turgid state only. It was CEDERVALL (**22**) who first so aptly described the collapsed walls as "accordion-pleated." Subsequently, most authors have compared the appearance and action of the folding (wrinkling [**13**]) and stretching of the cell walls to that of an accordion (**126, 167**).

This phenomenon explains, in part at least, why detached branches from the pineapple plant—the slips and shoots—and the crown from the fruit remain viable even after great desiccation, such as takes place with the curing of this propagating material before planting, and why their leaves regain their turgor with an adequate water supply after planting. It further explains why field plants recover from drought conditions which result in the death of adjacent nonxerophytic plants.

The water-storage tissue may well protect the underlying assimilatory tissue against excessive light (**22, 33**).

[23] SCHULZ (**126**) identified, through the use of proper dyes and their double refractive property, a lamella layering in ringlike "stiffening" zones on the walls of these cells in another species of Bromeliaceae; she believed that this modification in wall structure served to protect the tissues against the stresses involved in the collapse and extension of these walls.

CEDERVALL (22) believed that in Bromeliaceae this purpose was served by this tissue in both the turgid and the collapsed state. On the other hand, PFITZER (104) thought that this tissue served to absorb heat without appreciably reducing the light incident to the chlorophyllous assimilatory tissue beneath. CEDERVALL also believed that the water-storage tissue serves to stiffen the leaf as a result of the turgidity of its cells. It is true that the blade proper of the leaves of many Bromeliaceae (the pineapple leaf to a small degree) loses some of its stiffness or crispness and the margins curl inwardly when there is loss of water from the water-storage tissue, but the stresses involved in this direction are very slight in comparison with those in a direction parallel with the leaf axis.

The tissue occupying the upper portion of the non- and subchlorophyllous regions of the leaf is not distinct from the lower portion, as in the chlorophyllous region just described. In the nonchlorophyllous region the ground tissue—all tissue exclusive of the vascular bundles, the fiber strands, the aerating canals, the epidermis, and the hypodermal layer—consists of a fairly uniform parenchyma, there being little difference in size and shape of the cells in the upper and lower portions of the leaf (fig. 85). There does appear to be a little less rigidity to the cells of the upper portion. Examination of longitudinal sections shows that these cells are rather irregularly arranged spherical cells, whereas those in the lower portion are most often aligned in regular rows and, as a result, assume a more polyhedral form. The parenchyma cells in both upper and lower portions of this region of the leaf are usually filled with starch grains. There is no indication that any cells in this region serve as water-storage tissue in the sense that the term is used in the chlorophyllous region (126).

In sections cut in acroscopic succession in the subchlorophyllous region, the shape of the cells in the upper portion of the leaf varies gradually from that just described for the nonchlorophyllous region to that characteristic of the water-storage tissue in the chlorophyllous region. In the subchlorophyllous region, at least as the true chlorophyllous region is approached, this tissue assumes the function of water storage.

MESOPHYLL

The term "assimilatory" or "photosynthetic" tissue may be correctly used in connection with the pineapple leaf to include both the cells of the ordinary mesophyll, which occupies the greater portion of the lower half of the leaf thickness, and the stellate cells of the specialized mesophyll confined to the aerating canals but which also function in an assimilatory capacity. Since the aerating tissue has already been described, this description of assimilatory tissue will be confined to the mesophyll proper.

This mesophyll extends from the lower hypodermal layer to the water-storage tissue in the chlorophyllous region, interrupted only by the row of alternating vascular bundles and aerating canals near its upper limit and by the fiber strands scattered through it.

Two types of mesophyll cells have been reported to occur in bromeliaceous leaves. Early writers (22, 104, 110) described both isodiametric and palisade or palisade-like cells, while later authors (94, 141) wrote of the assimilatory tissue as rarely or never composed of palisade or palisade-like cells but entirely of spherical or polygonal cells. BERGMAN and WELLER (13) and CEDERVALL (22),

describing *A. comosus* specifically, merely described this tissue (called "chlorenchyma" by BERGMAN and WELLER and "chlorophyllous parenchyma" by CEDERVALL) as being made up almost entirely of isodiametric cells.

Examination of many pineapple leaves over a period of years has shown that the prevailing type of cells in the mesophyll of the chlorophyllous region is isodiametric and nearly spherical (figs. 120, 123*A*), typical of unspecialized mesophyll in other plants, with diameters of 0.5–0.9 mm. Those cells in the first layer, or first two or three layers, adjoining the water-storage tissue (figs. 119, 121) and several layers of cells immediately above the lower hypodermis in the ridges (fig. 122) are often, but not always, elongated perpendicularly to the leaf surface so that their height is about twice that of the shorter diameter (fig. 123*B*). Although these elongated cells somewhat resemble the familiar palisade cells of other plants in shape and arrangement, i.e., aligned in rather regular compact rows, these cells are not true palisade cells in the strictest sense of the term.

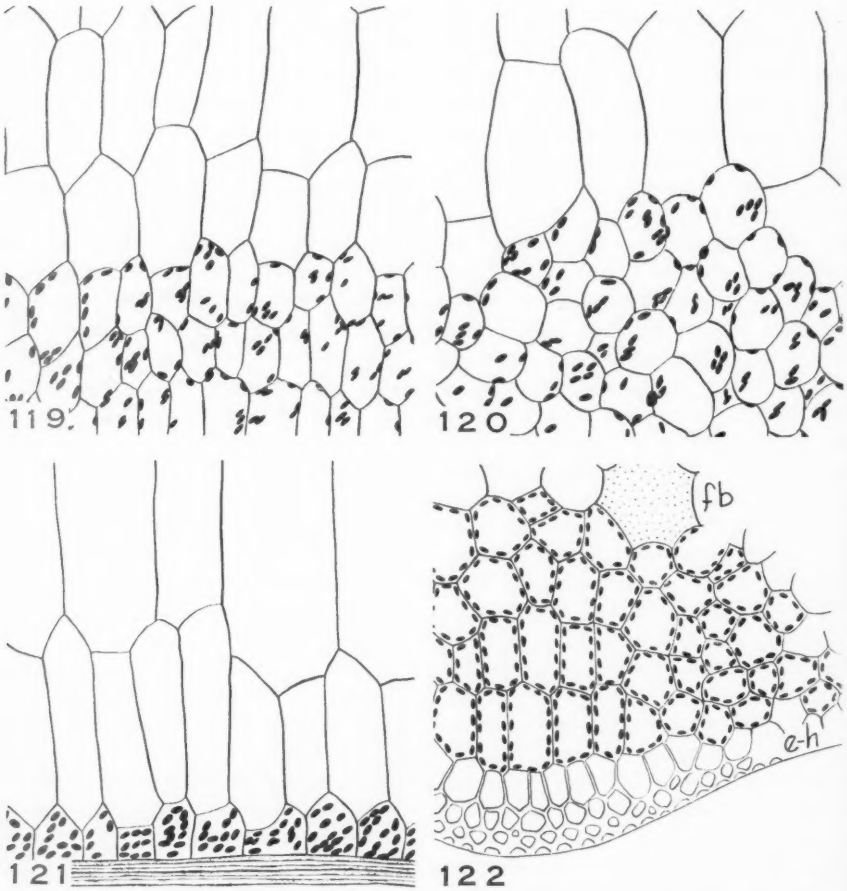
Both isodiametric and elongated cells have thin cellulose walls, and intercellular spaces are absent or very small. The cells contain few to many chloroplasts and often have few to many starch grains. The chloroplasts are usually ellipsoidal but range from that to discoidal or even spherical (figs. 123–125). They are usually arranged along the walls of the cells, so that in thin transverse sections of the leaf they appear in profile (figs. 122, 123*A*). This type of orientation of chloroplasts in leaves of plants exposed to intense sunlight has been noted repeatedly (**12, 33**). In the pineapple plant, however, the chloroplasts frequently appear scattered through the cell (figs. 121, 123*B*). This is usually the case when there are a large number of chloroplasts in the cells, but whether this is also associated with plants growing in areas of moderate as opposed to intense sunlight has not been determined.

In his study of *Tillandsia usneoides* (family Bromeliaceae), BILLINGS (**14**) described in detail a "peculiar" structure and behavior of the chloroplasts. These, which he called "megachloroplasts," are composed of masses of "smaller chloroplasts," designated as "microchloroplasts," instead of showing the more or less homogeneous structure observed in most chloroplasts. He found that the microchloroplasts do not remain conglutinate but become dispersed throughout the cells, where they show a lively Brownian movement. Suspecting that whether the chloroplasts remained in the megachloroplast form or became "divided up" into microchloroplasts was conditioned by light intensity, BILLINGS carried on a series of experiments which demonstrated only that both forms may occur in the same leaf under all conditions. The conception of this peculiar complex chloroplast has been maintained for a long time (**11, 141**).

In a study of the "aging" of pineapple leaves under various environmental conditions (unpublished data) some years before seeing BILLINGS' paper, the writer observed what appeared superficially to be conglomerate or complex chloroplasts. This apparent breaking-up of chloroplasts into smaller ones was not then studied further, but later it seemed worth while to examine more carefully the chloroplasts in the pineapple plant and several other members of the Bromeliaceae to see if these actually resemble those described by BILLINGS for *Tillandsia* and to determine the true nature of the smaller bodies (**78***a*).

Sections of fresh material from grow-

ing plants of both *T. usneoides* and *A. comosus* and also from two other bromeliaceous species, *Bromelia pinquin* and *Chevallieria stephanophera*, were examined at different times of day. It was found that the bodies which BILLINGS reported as "microchloroplasts," formed by the breaking-up of "megachloroplasts" in *Tillandsia*—a phenomenon which appeared to exist in the pineapple



FIGS. 119–122.—Water-storage tissue and mesophyll. Figs. 119–121, transition from water-storage tissue to mesophyll in chlorophyllous region of fully developed leaf. Figs. 119–120, longitudinal and transverse sections, respectively; note rather sharp, i.e., straight-line, boundary between water-storage tissue and mesophyll in fig. 119, and mesophyll cells projecting into water-storage tissue in fig. 120. Water-storage tissue occupies approximately half of leaf thickness; mesophyll the remaining half. In fig. 119 mesophyll cells are somewhat palisade-like; these are not usual in pineapple while the globose cells in fig. 120 are. In fig. 121 two fibers of isolated strand mark boundary of water-storage and mesophyll tissues; first row of mesophyll cells constitutes parenchymatous sheath. Fig. 122, transverse section of lower side of mature leaf to show two rows of palisade-like mesophyll cells in ridge beneath epidermal-hypodermal complex (*e–h*). *fb*, isolated fiber strand. ×200.

on superficial examination by the writer —are actually starch grains which are formed in the chloroplast and then become free from it. Drawings of chloroplasts from the four species observed at different times of day are shown in figures 124 and 125. Examination shows that the chloroplasts in the various species resemble one another closely in what this writer will call their "resting stage": they are ellipsoidal, and the material constituting them appears to have a definite structural arrangement (figs. 124*A*1, *B*1, 125*A*1, *B*1). SHARP (**129**), in a recent summary of the conceptions of chloroplast structure, stated that chlorophyll seems to be confined to numerous small platelets, or *grana*, imbedded in the stroma—a colorless cytoplasmic matrix. The "fibrous" appearance exhibited by chloroplasts is probably due to a linear

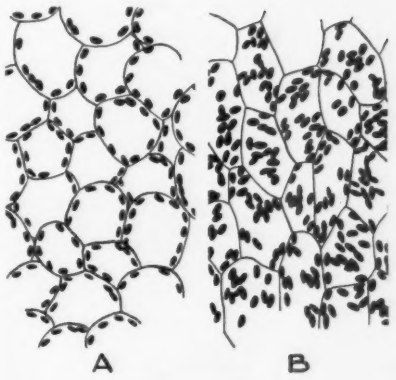
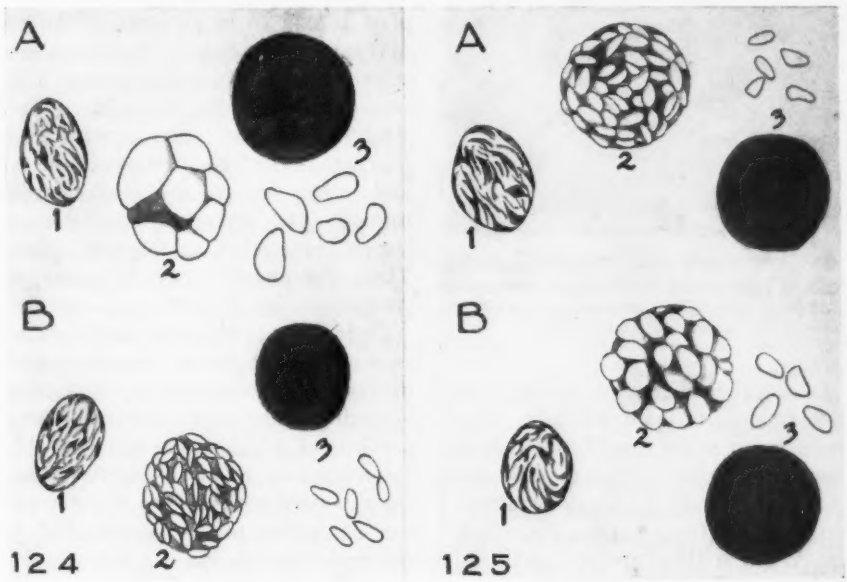


FIG. 123.—Chloroplast arrangement in mesophyll cells. *A*, isodiametric mesophyll cells, more usual form in leaf, with chloroplasts arranged along walls of cells, and *B*, less common, more elongate form of mesophyll cell, with chloroplasts scattered through cells. ×200.



FIGS. 124–125.—Chloroplasts. Phases of chloroplast structure during day. Fig. 124*A*, in pineapple; and in other bromeliaceous species: Fig. 124*B*, *Tillandsia usneoides;* Fig. 125*A*, *Chevallieria stephanophora;* Fig. 125*B*, *Bromelia pinguin*. In all species the following phases: 1, "resting stage"; 2, formation of starch grains in chloroplast; 3, starch grains free of chloroplast. ×1650.

arrangement of the grana and to variations in the submicroscopic structure of the stroma itself. It is possible that the configuration observed in the bromeliaceous chloroplasts examined is of the type reported by SHARP and by GUILLIERMOND (52) for chloroplasts in general.

FIG. 126.—Starch grains. Vertical longitudinal section of basal portion of seedling to show storage (secondary) starch as compound grains. Hilum visible in each individual grain. ×920.

During the progress of the day, probably dependent upon sunlight, starch grains begin to form within the chloroplast until the latter appears to be made up almost entirely of a conglomeration of starch grains (figs. 124*A2*, *B2*, 125*A2*, *B2*). Whether these are confined within a membrane cannot be stated at this time. Individual starch grains "break away" or "emerge" from the chloroplast and become free within the cell. They show lively Brownian movement. These starch grains occur singly.

When no starch grains remain within the chloroplast, the latter appears as a pale-green, sometimes hardly visible body, more or less spherical in shape. There appears to be a limiting membrane, as is now admitted for chloroplasts (129). The contents at this stage appear wholly homogeneous (figs. 124*A3*, *B3*, 125*A3*, *B3*). The possibility that the configuration noted in the "resting stage" (figs. 124*A1*, *B1*, 125*A1*, *B1*) may represent folds in the limiting membrane resulting from a collapse of the chloroplast shown in this third phase (figs. 124*A3*, *B3*, 125*A3*, *B3*), instead of some internal structural arrangement, should not be overlooked. Further study will be required before more exact descriptions and the processes involved can be given.

The starch synthesized in the chloroplast is said to be "primarily" formed (33) and is known as "assimilation" starch (128). In the pineapple leaf it appears to be rapidly transformed to a soluble compound; excess quantities of this pass to the basal portions of the leaf and stem, where the "secondarily" formed (33) "reserve" or "storage" starch (128) arises in the amyloplasts. These "secondary" starch grains are compound (figs. 45, 126).

As indicated previously in the section on water-storage tissue, there is no sharp distinction between the types of tissue occurring in the upper and in the lower portions of a leaf in the non- and subchlorophyllous regions. Transverse sections in these regions show that the portion equivalent to the mesophyll of the chlorophyllous region consists of cells which in shape somewhat resemble the isodiametric mesophyll cells but are often larger and without chlorophyll except in the parenchymatous sheath cells

and in the cells bordering the secondary aerating canals, as stated previously. In many cases, however, they are less nearly spherical than the true mesophyll cells, being isodiametric in paradermal sections but more elongated perpendicular to the surface. These cells are usually filled with compound starch grains of the storage type; often the grains seem to fill the cell lumen completely. MEZ (**94**), writing of Bromeliaceae in general, noted that the largest portion of the starch reserve in leaves is deposited in the basal ensheathing portions.

HYPODERMAL LAYER

The terminology used by various early authors in the description of the hypodermal layer as it occurs in bromeliaceous plants is, unfortunately, very confused. Some authors (**57, 116**) wrote of the epidermis and what we now call the hypodermal layer as parts of a multiple epidermis and so named it. Others (**30, 88, 104, 110, 141, 159**) properly located and described this tissue but used the term "hypodermis," "hypodermal-layers," "-tissues," or "-ring" to include both the true hypodermal layer and/or the water-storage tissue. MEZ (**94**) and VON MOHL (**99**) also described and located these cells correctly, but MEZ wrote that they must be considered as part of the mesophyll and not as a hypodermis; VON MOHL did not name the tissue. Several authors describing *A. comosus* (**13, 22**) and other bromeliaceous species (**126, 167**) have used the term "hypodermis" correctly in the modern sense (**33**). MILES THOMAS and HOLMES (**98**) reported no hypodermis in the pineapple seedling leaf; it does not occur there.

In the fully developed pineapple leaf the hypodermal layer consists of from one to several rows of sclerenchyma cells lying beneath the epidermis; the number of rows varies with their position in the leaf.

In the basal, least mature portion of the leaf the hypodermal cells are identified by their position, immediately beneath the epidermis (figs. 127–130), rather than by those distinguishing features which characterize them in more mature regions. In the subchlorophyllous and chlorophyllous regions, the hypodermal layer consists of a single row of cells on the upper side and of a single row in the furrows and of two rows in the ridges on the lower side (figs. 122, 133). At the leaf margins in the chlorophyllous region the hypodermal layer consists of four to six rows of cells (fig. 96), and at the tip it includes three to five rows (fig. 97). This characteristic thicker hypodermal layer in the ridges of the lower side and at the margins of the leaf had been previously noted in *A. comosus* (**13, 22**) and other bromeliaceous species (**30, 141**).

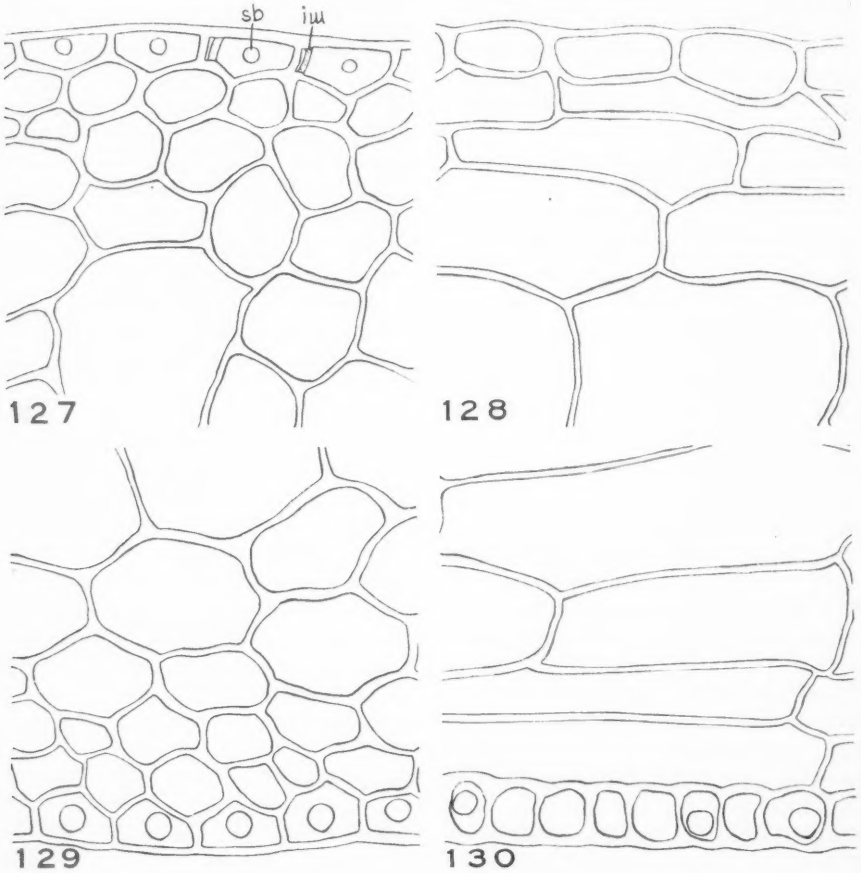
Beneath the rows of definitely hypodermal cells there is usually an additional row which may correctly be included in the hypodermal layer. The walls of these cells are not so thick as the hypodermal cells immediately beneath the epidermis, but they are markedly thicker than the walls of the adjacent parenchyma cells (figs. 122, 133). The thickening is especially pronounced at the corners, giving these cells the appearance of collenchyma cells in cross section (fig. 133). RICHTER (**110**) called this row of cells in *A. comosus* "collenchyma," but these cells are not true collenchyma.

With the exception of those in the furrows, the hypodermal cells are essentially alike in shape and size in the various portions of the leaf; they are smaller at the margin and tip. The thickness of the wall, however, varies with the leaf region and with age (figs. 127–134).

The cells are elongated in the direction

of the leaf axis, with the end walls usually oblique, or ever tapering in some cases; often, however, they are truncate, or at right angles to their radial walls (figs. 131, 132, 134). Similar descriptions of the general shape of these cells in *A. comosus* have been presented by previous writers (13, 22).

The shape of the hypodermal cells can be seen to best advantage in paradermal sections in the more immature regions of the leaf, where the inner walls of the epidermal cells have not yet become thickened and the cells of the first hypodermal row are visible through the epidermis. In cross section these cells appear



FIGS. 127–130.—Epidermis and hypodermal layer. Figs. 127, 128, transverse and vertical longitudinal sections, respectively, of *upper* side, and figs. 129, 130, transverse and vertical longitudinal sections, respectively, of *lower* side of basal portion of fully developed leaf, to show layers before thickening of cell walls. Note relative lengths of longitudinal and transverse walls of epidermal and hypodermal cells. Most of silica bodies (*sb*) lost in cutting vertical longitudinal sections. Apparent doubling of wall at *iw* in fig. 127 is result of undulation. ×650.

as four- to six-sided polygons (figs. 127, 129). When mature, they have the appearance of stone cells in such a section (fig. 133); although the hypodermal cells in some bromeliaceous plants are reported to be stone cells (**141**), they are not in the pineapple. In the latter plant they represent a transitional type between the typical fiber—such as occurs in the isolated fiber strands and in the sclerenchyma sheaths of the vascular bundles—and typical stone cells; they might be called short, stone-cell-like fibers or elongate stone cells, as EAMES and MACDANIELS (**33**) have suggested for other plants.

In the mature portions of the leaf the cells in the first hypodermal row, especially, are characterized by extremely thick walls (figs. 133, 134). These walls, originally cellulose, soon become lignified and, when greatly thickened, cutinized for the greater part. This can be shown very nicely by staining with the SCHNEIDER-ZIMMERMANN (**122***a*) triple stain. Because the complex made up of the inner walls of the epidermal cells and the outer walls of these hypodermal cells gave a cutin reaction, VON MOHL (**99**) wrote of a "cuticle" lying between two rows of cell lumens (those of the epidermis and first hypodermal row).

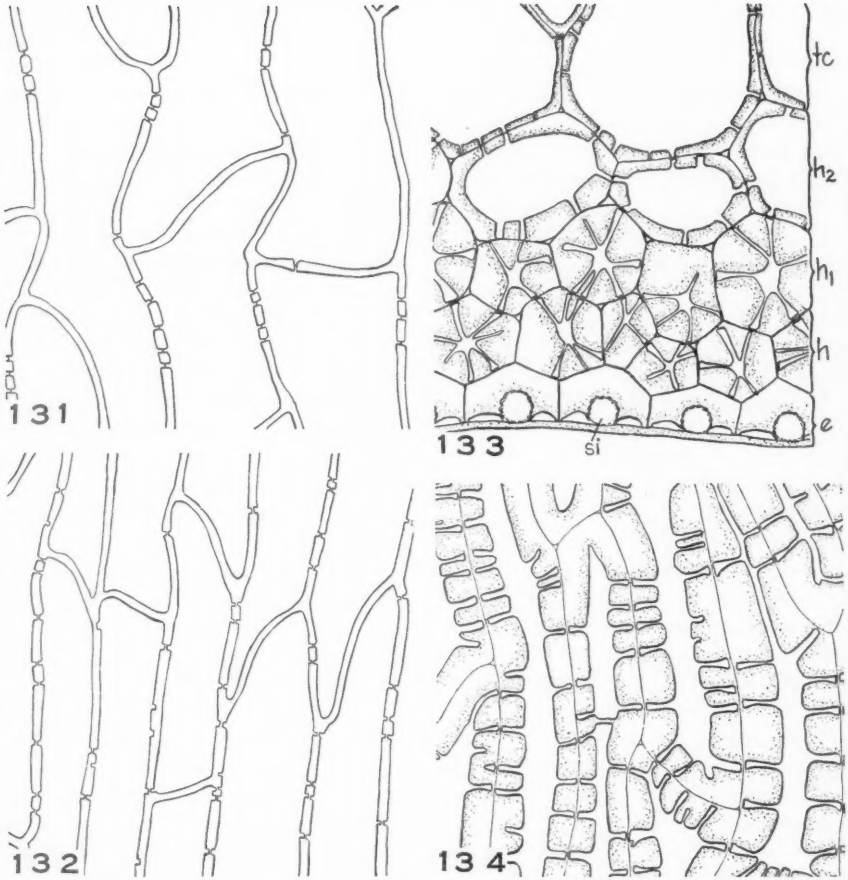
The thick walls of the first row of hypodermal cells in the pineapple leaf are profusely pitted, as they are generally in Bromeliaceae (**13, 22, 94, 141**). A striking feature of these pits in the pineapple, and noted in other Bromeliaceae (**22, 104, 110**), is that the pore canals in these hypodermal cells do not have complementary canals in the adjacent epidermal cells. In dicotyledons such uncomplementary pits occur in secondary xylem and are called "blind" pits (**33**). The pits in the pineapple hypodermal cells are of the simple type.

The cells in the second hypodermal row in the ridges on the lower side of the leaf and in the second to sixth row at the margin are practically identical with those described for the first row (fig. 133).

As stated previously, the row or rows of collenchyma-like cells beneath the typical hypodermal cells, described above, are considered part of the hypodermal layer. These cells are usually of the same general shape as the typical hypodermal cells but have greater diameters in successive rows. Their walls are not so thick, the thickening being greatest at the corners, as in typical collenchyma cells. The walls are lignified and usually pitted, the pore canals connecting with complementary canals in the first hypodermal row and with successive collenchyma-like layers (fig. 133).

When mature, the hypodermal cells appear without content; at no time do they contain chloroplasts. The collenchyma-like hypodermal cells contain a clear colorless cell sap, except for occasional cells on the upper side which contain anthocyanin. In aggregate the colored cells produce the "suffusion" of red in the form of a central streak along the length of the exposed portions of the upper surface of older leaves. Several authors (**22, 46, 59, 141**) have written briefly about anthocyanin in bromeliaceous leaves.

As in most plants (**33**), the hypodermis originates from the subepidermal ground tissue. PFITZER (**104**) described in some detail the ontogeny of this tissue in Bromeliaceae. Very early in the young leaf the cells lying directly below the epidermis first divide tangentially, parallel to the leaf surface. The outer daughter cells thus formed divide radially to become the first row of the hypodermal layer. The inner daughter cells formed by



FIGS. 131–134.—Epidermis and hypodermal layer. Fig. 131, paradermal longitudinal section of upper side of basal portion of fully developed leaf to show peculiar broadening of hypodermal cells near and at end—transverse—walls, sometimes found on this side of leaf. Note also tapering at ends of some cells. Cell walls not yet fully thickened; note pits. Fig. 132, paradermal longitudinal section through ridge on lower side of basal portion of fully developed leaf to show both truncate and oblique—tapering—end (transverse) walls and pits in hypodermal cells. Fig. 133, transverse section through ridge on lower side of mature leaf in chlorophyllous region to show epidermal and hypodermal cells. Note comparatively thin outer wall (chiefly cuticle), thick lateral and inner walls, and silica bodies (*si*) in epidermal cells (*e*); thick walls of cells in first (*h*) and second (h_1), and collenchyma-like cells in third (h_2), hypodermal layers; and transitional cells (*tc*). Pits prominent in all hypodermal and transitional cells. Fig. 134, paradermal longitudinal section through ridge on lower side of subchlorophyllous, transition region of mature leaf to show cells of first hypodermal layer (*h* in fig. 133). Note thick walls, middle lamella, and pits. ×650.

the first tangential division themselves divide first tangentially and then radially to form two more rows of hypodermal cells. This process continues until the designated number of hypodermal cell rows has been formed. On the upper side of the pineapple leaf several additional series of tangential and radial divisions result in several rows of transitional cells between the collenchyma-like hypodermal cells and the water-storage cells. On the lower side of the leaf the first and only row of collenchyma-like hypodermal cells is immediately adjacent to the mesophyll cells, with no transitional types present except in the ridge where there is a row of cells of transitional type without chloroplasts (fig. 122).

The hypodermal layer may be considered to serve two functions. Mechanically, it must serve an important role in stiffening and strengthening the long, narrow, and comparatively thin leaf. It therefore supplements the mechanical function of the isolated fiber strands and sclerenchyma sheaths of the vascular bundles. That the wedge-shaped lower surface of the inner wall of the epidermal cells fits intimately between the cells of the first row of hypodermal cells (see next section) also may be considered to serve a mechanical purpose. The hypodermal layer also provides, along with the thick-inner-walled epidermis, a protection against water loss, as in other xerophytes.

The additional rows of hypodermal cells at the margins of the leaf serve to protect it against shearing; HABERLANDT (57) termed this property of a leaf to resist shearing "shear-resistance" (*Schubfestigkeit*).

The marginal extension of the hypodermal layer in the spines will be described in a later section.

EPIDERMIS

The epidermis is considered here as a structural unit, i.e., the mass of epidermal cells and their modifications, together with the epidermal appendages. This is the more modern treatment of the subject.

The epidermis of the leaf, together with its structural modifications and appendages—the stomata, the scalelike trichomes, and the simple multicellular filiform hairs (which occur only in seedling leaves)—forms one of the most, if not the most, striking and characteristic features of the pineapple plant and, in fact, of bromeliaceous plants in general. More literature has been published on this subject than on any other structural feature of these plants.

The epidermis proper consists of a single layer of cells. Most authors (**13, 22, 88, 110, 167**) describing this tissue in representatives of this family have written of or illustrated it as such. HABERLANDT (**57**) and SCHACHT (**116**), on the other hand, unfortunately depicted it as a multiple epidermis, including the first row or rows of the hypodermal layer with the epidermis. The ontogeny of the hypodermal layer, as outlined previously, proves that a multiple epidermis is not to be considered here.

CELL FORM.—In most narrow linear leaves, especially of monocotyledons, the epidermal cells are obviously elongated in the direction of the leaf axis except in a few plants including the Bromeliaceae (**30, 57**). In the latter the greater cell diameter is at right angles to the leaf axis—transversely, in the direction of the leaf width. It is strange that such a marked variance from the usual should not have drawn the attention of the many other investigators of the Bromeliaceae. These authors (**12, 13, 88, 94, 98**)

described or illustrated the epidermal cells of bromeliaceous leaves, including those of *A. comosus*, as elongated in the direction of the leaf axis, or through ambiguous descriptions gave this impression.

Examination by the writer of the epidermis in different regions of any one leaf, on leaves of different stages of development, and on leaves from plants of different ages has shown that in the more mature regions of leaves from older plants the cells appear, for the most part, elongated at right angles to the leaf axis (figs. 135, 138, 139). The longitudinally elongated—in the direction of the leaf axis—type of cell does occur, however, primarily in the leaves of seedlings and at the very base of growing leaves from older plants (figs. 136, 137, 142–144).

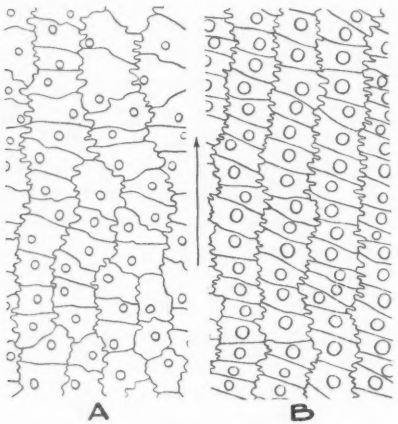


FIG. 135.—Epidermis. *A*, upper and *B*, lower epidermis of basal portion of half-grown leaf of 6-month-old plant. Note greater irregularity in shape of cells, greater dimension in direction of leaf axis (indicated by arrow), and smaller silica bodies in upper (*A*) than in lower (*B*) epidermis. ×300.

Although this correlation between form type and state of development of plant or leaf is rather general, it is a tendency rather than the rule. Thus in the mature chlorophyllous region of a fully developed leaf or in the young or more mature portions of the nonchlorophyllous region of a growing leaf, the epidermal cells on the ridges of the lower side are often more elongated in the direction of the leaf axis, while those in the furrows are more elongated in the direction at right angles to this (fig. 146); or the same relationship may exist between the epidermal cells of the upper and lower sides, respectively, in the basal portions of a fully developed leaf (figs. 135, 136, 138). The epidermal cells at the margins in the mature regions of the leaf are usually elongated longitudinally. Even in those regions where cells are more elongated longitudinally, the shorter type also occurs. The epidermal cells surrounding the stomata and trichomes are very irregular at leaf maturity; they will be described later.

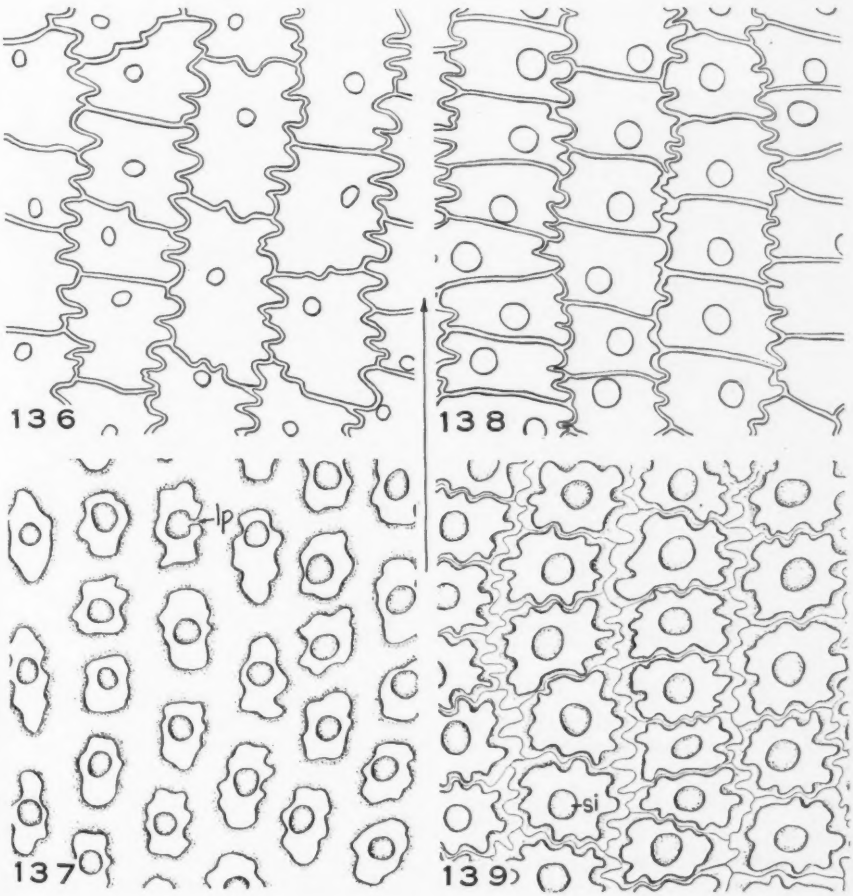
Except for the cases noted above, the general tendency for the epidermal cell in the pineapple leaf, therefore, is to be approximately rectangular in shape in surface view and in transverse section, with the greater diameter in a direction at right angles to the leaf axis. In longitudinal section they appear more nearly square.

CELL WALLS.—In the very early stages of cell development the walls are thin and composed of cellulose, and those facing each other—the two pairs of lateral walls and the outer and inner walls—are approximately parallel. With maturation, however, the lateral walls become undulate, the inner wall becomes thickened in such a way that its upper surface[24] becomes concave, and the outer

[24] To avoid confusion in the use of terms "upper" and "lower" surface of the inner wall, the description in the following paragraphs will be of epidermal cells on the upper side of the leaf. Epidermal cells on the lower side of the leaf resemble those described for the upper epidermis in all respects except as noted.

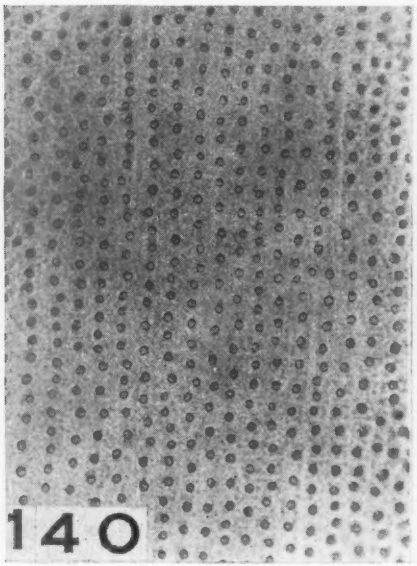
wall becomes slightly convex owing to the presence of the silica body. These features will be described later.

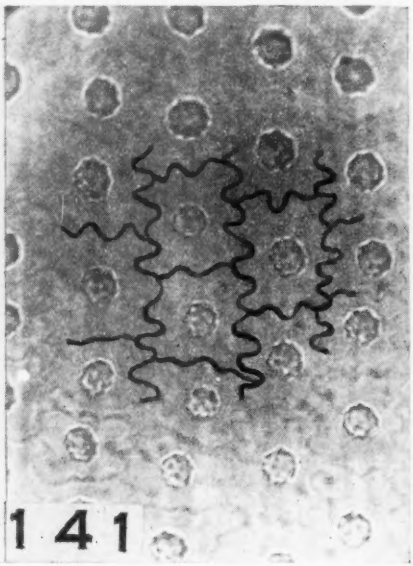
In seedling leaves and in the basal portions of young leaves of older plants all the walls of the epidermal cells are smooth, i.e., straight or flat (figs. 142–144, 146–149). This explains why MILES THOMAS and HOLMES (98), in their description of leaves of the pineapple seed-



FIGS. 136–139.—Epidermis. Figs. 136, 138, surface views of epidermis of basal, nonchlorophyllous region of fully developed leaf; fig. 136, on upper, and fig. 138, on lower side of leaf. Note cells are elongated in direction of leaf axis (indicated by arrow) on upper side, and in direction at right angles to this on lower side of leaf. This relationship of orientation of epidermal cells and leaf axes is not usual (see fig. 137). Fig. 137, surface view of epidermis on ridge on underside of chlorophyllous region of mature leaf. Note greatly thickened walls with undulated contour of original cell wall smoothed out; middle lamella not visible. Fig. 139, surface view of epidermis on ridge of underside of subchlorophyllous, transition region of fully developed leaf. Note greater thickening of longitudinal walls, and middle lamella; also tendency of thickened wall to have margins less undulated than original wall (middle lamella). Verrucose surface of silica bodies (*si*) not shown; these bodies sometimes connected to cell wall by process (*lp*). ×650.

ling and the young plant up to one year of age, made no mention of the undulate lateral walls of epidermal cells which are so characteristic of *Ananas*. The epidermal cells in the more mature regions of young leaves of older pineapple plants and in all regions of mature leaves, however, are characterized by undulate or not always the case. Often, in mature leaves, where the walls are very thick, they appear only slightly undulate (figs. 137, 139). On tracing the middle lamella, however, one sees that this layer—the original cell wall—is deeply undulate (fig. 139). This middle lamella may often be observed by examination of these cell



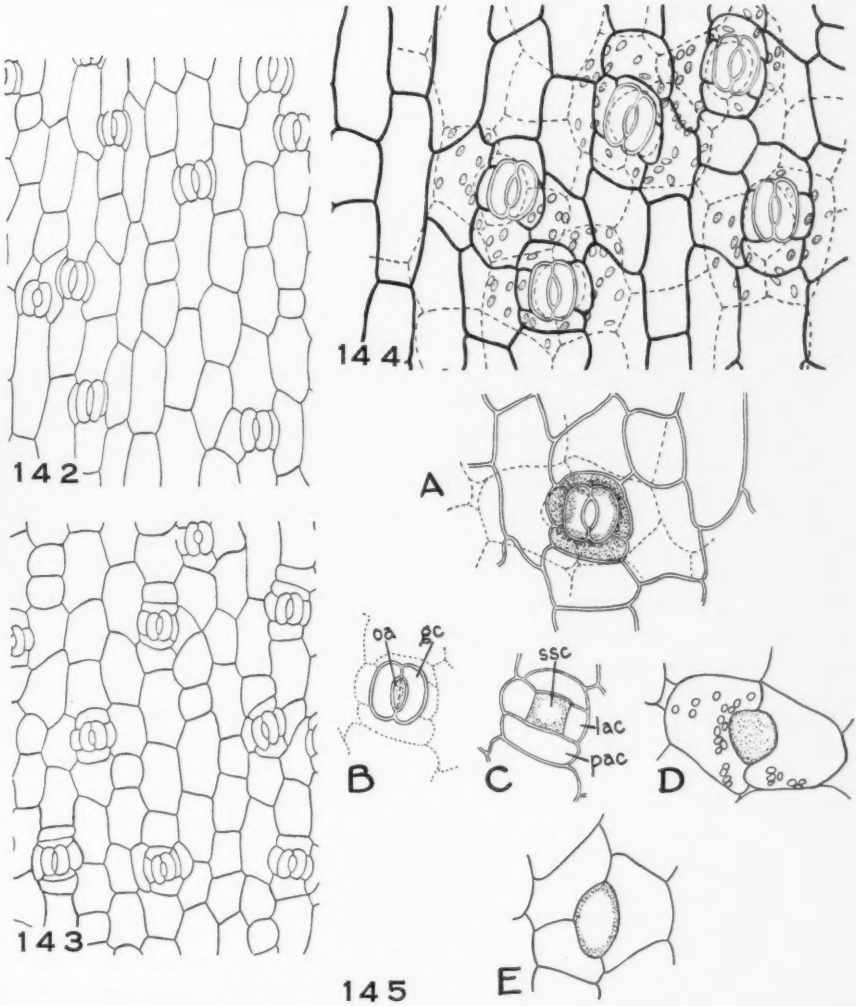


FIGS. 140–141.—Silica bodies. Surface views of basal portion of fully developed leaf to show silica bodies, one of which occurs in each epidermal cell. Silica bodies stand out as bright spheres, aligned in fairly regular rows. Fig. 140, under low power, ×200; and fig. 141, under high power, ×800, to show highly refractive quality and verrucose surface of bodies. Walls of several cells traced with ink to show undulation.

sinuate lateral walls. This feature was reported as general throughout the family Bromeliaceae (**22, 94**), while LINSBAUER (**88**) wrote that both undulated and smooth walls exist in mature cells of bromeliaceous leaves.

In the pineapple leaf the undulation extends equally over the whole height of the wall. Usually the longitudinal walls are more undulate than the transverse walls (figs. 135*A*, 136) or are the only undulate ones (figs. 135*B*, 138), but this is walls under high magnification; it is distinguishable from the thickening layers because of its refractive power. LINSBAUER (**88**) was able to show the middle lamella with the use of iodine-zinc chloride or special stains, or to isolate it, in *Ananas*, by dissolving away the thickening layers with concentrated sulfuric or chrom-sulfuric acids.

The "smoothing-out" of the walls, described above, results from the nonuniform thickness of successive thickening



FIGS. 142–145.—Stomata. Figs. 142, 143, surface views of epidermis on underside of seedling leaves to show elongated epidermal and guard cells in early-formed, fig. 142, and shorter cells in later-formed, leaf, fig. 143. Fig. 144, surface view of lower side of subchlorophyllous region of longest leaf from 3-month-old seedling to show stomatal apparatus and subepidermal cells (latter cells around substomatal cavity contain chloroplasts). Fig. 145*A*, surface view of individual stomatal apparatus with accessory cells shown stippled (lighter stippling indicates parts of accessory cells arching under guard cells). Hypodermal cells shown with broken lines. 145*B–E*, component parts of stomatal apparatus at successively lower levels from leaf surface: *B*, at level of guard cells which project slightly above level of epidermal cells (shown with dotted lines); *C*, at level of accessory cells; *D* and *E*, at level of substomatal ring (in *D*, two; in *E*, three cells form ring). Ring cells in *D* contain chloroplasts. *oa*, outer aperture; *gc*, guard cell; *ssc*, substomatal chamber; *lac*, lateral, and *pac*, polar accessory cells.

layers. Often, in those places where the undulation is such that the space between the folds is narrow, the thickening layers apparently fuse with one another secondarily. In the early stages of this fusion only the outer edges of the folds fuse so that, in a surface view of the epidermis, the wall appears perforated in places, or pitted. With further thickening, however, the fusion becomes complete, and the wall appears solid. LINSBAUER (88) had previously reported especially fine examples of this secondary type of fusing in species of *Billbergia* and *Cryptanthus*. He also stated that the thickening of the lateral walls of epidermal cells in leaves of an *Ananas* species often proceeds to the point where the thickness of the wall is greater than the diameter of the lumen (see fig. 137 for an example in the pineapple leaf).

DEBARY (30) has written that within one and the same species of plant the degree of undulation of cell walls varies with environmental conditions—there are notably smoother walls in dry, and more undulated walls in moist, regions. Whether the degree of undulation of epidermal cell walls of pineapple varies with environmental conditions is beyond the scope of this paper. All that can be said is that the pineapple, classed as a xerophytic species, is characterized by undulate walls. It would be interesting to determine the degree or lack of undulation in the walls of epidermal cells of various bromeliaceous species in correlation with their natural habitat.

That the undulation of the longitudinal walls, primarily, and of the transverse walls, secondarily, serves a mechanical function seems very likely. The epidermis of a leaf of the pineapple type —thin, narrow, and very elongate—is constantly subjected to a variety of tensile stresses. Under these circumstances the firm attachment of one epidermal cell to its neighbors becomes especially important. In writing of plants in general, or specifically of bromeliaceous plants, several authors (**22, 57, 94**) have commented on the additional stiffening and strengthening brought about with the increased surface contact between adjacent elements in the case of undulate walls. The "fitting-together" of undulate walls of adjacent cells has aptly been compared with the sutures of the vertebrate skull or with "meshed gears." When the undulation extends the whole height of the epidermal cell, as it does in pineapple, such rigidity of the entire epidermal cell results as to protect it from the great stresses resulting from the collapse of the water-storage tissue beneath during periods of drought.

While the lateral walls are thickening, the inner wall also becomes greatly thickened. This latter feature was found to be so general for the epidermal cells of Bromeliaceae that it was considered by many investigators (**14, 22, 88, 94, 110**) to be one of the most important anatomical characteristics of this family. Although some of these authors found several exceptions to the occurrence of a thicker inner (than outer) wall in the epidermal cells of Bromeliaceae, the greater number of species do show this characteristic development of cell walls, and in the pineapple, to an especially high degree.

It is interesting to consider theories of the function of this thick inner wall. HABERLANDT (**55**) presented an interesting explanation which has, however, little basis in the light of ontogenetic studies of the pineapple. He has described a "relationship" of a protective covering of trichomes and thin epidermal cell walls in young leaves of *Hechtea* changing to one where the trichome heads are dry and loosen from the surface of the older

leaf and the inner walls of the epidermis become greatly thickened as a casual phenomenon—whereas actually this change is a simple maturation process.

HABERLANDT (**57**) listed among the functions of epidermal cells—and, more specifically, the outer epidermal wall—of plants in general, the following: protection of the tissue beneath against (*a*) desiccation, (*b*) mechanical injury, (*c*) excessive illumination or loss of heat by nocturnal radiation, and (*d*) violent fluctuations of temperature; to these may be added a fifth function, that of water storage, suggested by WESTERMAIER (**159**). In most Bromeliaceae, and in the pineapple specifically, the functions mentioned by HABERLANDT are assumed by the thick inner rather than the outer epidermal wall, which is comparatively thin. The function of water storage (**159**) can apply only in the early developmental stages of epidermal cells; later this function is assumed by the water-storage tissue (**57, 88**).

Several authors (**88, 110, 167**) believed that the degree of thickening of the inner wall of epidermal cells of the Bromeliaceae varies with the natural habitat of the various species. They found that this wall was thickest in the species growing chiefly in very dry habitats and concluded that this thick wall serves to protect the internal tissues of the leaves in such unfavorable environments.

With thickening, the upper surface of this inner wall becomes concave and the lower surface wedge-shaped (fig. 133). These two characteristics of the inner wall of the pineapple epidermal cell are also evidently characteristic of many other bromeliaceous plants, according to the literature.

BAUMERT (**12**) conceived of this concavity of the upper surface of the inner wall of the epidermal cells of many Bromeliaceae as a "concave mirror-like" structure and believed that, as such, it served as a light reflector, protecting the underlying tissues against excessive light and temperature. As partial confirmation of his theory BAUMERT noted that, on "fluted" leaves—concave in transverse section—of the Bromeliaceae he studied, this type of inner wall surface was best developed in those epidermal cells in the central portion of the leaf, where the rays of light fall perpendicularly during the brightest part of the day.

HABERLANDT (**57**) agreed with BAUMERT's theory, whereas LINSBAUER (**88**) did not. The latter believed that whatever reflective quality the upper surface of the inner wall might offer was completely counteracted by the outer wall and cuticle and by the trichome heads when present. In fairness to BAUMERT it should be stated that he was describing those bromeliaceous leaves without trichomes on the upper-leaf surface. LINSBAUER conceded that BAUMERT's reasoning regarding the illumination of fluted leaves would be correct if the leaves were set approximately horizontal; bromeliaceous leaves, however, in most cases stand more or less steeply upright and, besides, curve backward, so that the difference between the illumination of the medial and marginal portions of a leaf, during the brightest part of the day, especially, is of no moment.

Although the upper surface of the inner wall of the epidermal cells of pineapple leaves is definitely concave, it seems most unlikely to this writer that this surface serves usefully as a light reflector, even on the upper surface of the leaf, where the trichome heads are fewer in number, not only because of the reasons already presented by LINSBAUER but because of the presence of silica bodies in the epidermal cells. This crys-

talline inclusion body may itself serve as a reflector, but in mature cells especially, where it occupies the greater part of the lumen of an epidermal cell, as it does in the pineapple, there can be no thought of light reflection from the inner wall.

That the concave surface of the inner wall does indirectly serve a function is likely. The concavity results from a greater thickening of the lateral walls toward the inner wall (fig. 133). Thus in a transverse section of a leaf the lateral walls appear to have a broad base. Some authors (**88, 159**) likened a series of such "broad-based" walls to a system of groins attached to the hypodermal layer and stressed the mechanical strength that such a system provided.

As previously mentioned, the lower surface of the inner wall is wedge-shaped; this feature serves a mechanical purpose also, since this wedging of the cells of the epidermis between the cells of the first row of the hypodermal layer makes for an unusually intimate contact between the two layers. LINSBAUER (**88**) demonstrated this structural feature, which he called a thick membrane complex, in various Bromeliaceae with the aid of an appropriate stain or by maceration. In the pineapple the structure may be observed under high magnification without staining or other treatment (fig. 133).

LINSBAUER considered this wedging of the one layer of cells with the underlying one as a counterpart of the dovetailing of the epidermal with the hypodermal cells which VON HÖHNEL (**57**) demonstrated in the awns of certain Gramineae. Actually, the type of attachment of the epidermal to the hypodermal layer is different in the representatives of the two families. In the pineapple the lower surface of the inner wall of an epidermal cell, although wedge-shaped, is smooth, while in the grasses described by VON HÖHNEL the inner wall of the epidermal cells and the "outer" wall of the hypodermal cells present a serrated appearance owing to the presence of numerous lateral protuberances on the walls of the latter which fit into corresponding depressions in the former.

It is evident that the means of union between the epidermis and the underlying row of the hypodermal layer, and between the successively deeper rows of hypodermal cells which are similarly wedged (fig. 133), establishes a type of structure of considerable mechanical importance. The tensions to which the complex of epidermis and hypodermal layer is subjected through the accordion-like movement of the underlying water-storage tissue, and the movement of the leaf as a whole, necessitate an intimate contact between these layers in order to avoid a separation of the two tissues (**88**).

The outer wall of the epidermal cells of pineapple leaves is very little thickened, being the least thick of all the walls. This outer wall lies more or less flat except when the cell is mature; then, because of the size of the silica inclusion body within the cell lumen, the outer wall often becomes slightly convex (fig. 133).

CUTICLE AND CUTINIZATION OF CELL WALLS.—Reports on the presence of a cuticle and of the cutinization of the epidermal walls in Bromeliaceae have varied greatly. VON MOHL (**99**) wrote that, in general, thin leaves with thin outer walls possess a cuticle, whereas in thick, fleshy or leathery leaves with thick outer and lateral walls there is a cutinization of these walls as well as a cuticle present. He found an exception to these relationships in *A. comosus*, with its thinner outer and thicker inner walls; in this species he reported an "inner cuticle" as lying between the epidermis and first hypo-

dermal layer. VON MOHL mistook the cutinized wall complex made up of the thick inner walls of the epidermal cells and the thick outer walls of the underlying hypodermal layer for a cuticle. Several authors (**104, 110, 165**) recognized that to call this complex a "cuticle" was incorrect but did not identify these walls as cutinized. They and others described various bromeliaceous epidermal cells as possessing a cuticle but did not mention cutinized cell walls (**88, 117**) or cutinized outer walls (**13**). Some authors (**76, 141, 163, 164**) reported a "protein reaction" when the walls were tested with Millon's reagent or other chemical reagents, while FISCHER (**41**) denied this.

In the light of the writer's investigations previous interpretations have been either incomplete or erroneous. The entire surface of the epidermis of a mature pineapple leaf is covered with a layer of cuticle. Since the outer walls of the epidermal cells are very thin, this cuticular layer is often as thick as the original wall, as LINSBAUER (**88**) has observed in other Bromeliaceae. The upper surface of the cuticle is smooth, there being no evidence of the peculiar cuticular pattern —the presence of small undulations, forming bands—reported for another Bromeliaceae (**88**). SOLEREDER and MEYER (**141**) have remarked that "cuticular striation" is rare in Bromeliaceae. By use of proper stains[25] it may be seen that, besides the presence of a cuticle, the outer, lateral, and inner walls of the epidermal cells of the pineapple leaf are cutinized. Originally cellulose, they very soon become lignified and at maturity are cutinized. The cutinization of the walls of the hypodermal cells has already been discussed.

No wax covering is present on the pineapple leaf such as is reported for other Bromeliaceae (**30**).

SILICA BODIES.—In early stages of epidermal cell development—before decided thickening of the walls takes place —these cells contain a living protoplast which is colorless and lacks both chloroplasts and starch grains. By the time the walls have thickened, however, the cells are devoid of contents except for the peculiar silica bodies. LINSBAUER (**88**) was the first to demonstrate that these bodies in Bromeliaceae are composed of silica; he used accepted methods for silica identification. This writer has repeated these tests on the silica bodies in pineapple leaves.

It is strange that, with the exception of LINSBAUER—who found silica bodies in epidermal cells of all bromeliaceous leaves he studied, with few exceptions— BAUMERT (**12**), BULITSCH (**20**), and HARTWICH (**58**), none of the investigators (**22, 94, 110, 166, 167**) who have examined and reported on the epidermis of bromeliaceous leaves mentioned this prominent anatomical feature. BERGMAN and WELLER (**13**) illustrated but did not describe the bodies in pineapple. KOHL (**75**) erroneously reported the Bromeliaceae as one of the few monocotyledonous families lacking siliceous bodies. LINSBAUER (**88**) reported that the size of silica bodies varies with the species but that the variation is not great. He found the largest in *A. comosus* and a *Dyckia* species; these had diameters of 3 μ and up. The writer has measured silica bodies in pineapple leaves with diameters of 3–5 μ.

Silica bodies do not appear in the leaves of pineapple seedlings and so were unreported by MILES THOMAS and

[25] If freehand transverse sections are stained with a SCHNEIDER-ZIMMERMANN (**122a**) triple stain—fuchsin, picric acid, and methylene blue—a beautiful differentiation of cutinized, lignified, and cellulose walls is obtained.

HOLMES (98). In older plants they form at an early stage in leaf development. They are, at first, very small and irregular in shape, appearing as a single crystal. With increase in cell size and development, the silica body increases in size, owing evidently to further deposits of silica on the original body. There is a tendency for the bodies to become more nearly spherical with increase in cell age, although ovoid bodies are not entirely uncommon, even in mature cells. When the cell walls become very thick and the silica body very large, the latter often fills the entire lumen of the epidermal cell and thus appears imbedded in the inner wall (fig. 133). LINSBAUER (88) was reminded somewhat of the stegmata in the leaves of palms, but KOHL (75) definitely proved to his own satisfaction that there are no stegmata in Bromeliaceae.

The silica bodies are highly refractive, and in a surface view or a transverse section of the epidermis they stand out as rows of shiny spheres (fig. 140). The bodies do not have a smooth surface (although so pictured in the drawings in this paper, for simplification, but, as described by LINSBAUER (88), have a fine warty-like surface (fig. 141). This structural feature might lead to the opinion that the body represents a conglomerate of tiny silica crystals (as it may, to be sure), but examination of bodies at various stages of cell development suggests that the silica is deposited in successive layers, perhaps in organic combination. It was beyond the scope of the present investigation to determine the exact chemical composition of these bodies. That there is some crystallization of the silica is indicated by the rough surface.

Sometimes the single silica body which occurs in each epidermal cell is connected to a lateral cell wall by a process projecting from the latter (fig. 137, at *lp*).

Any opinion on the function of the silica body can be only conjecture at best (88). BAUMERT (12) believed that its one and only function might be to serve in light dispersion because of its location and structure but admitted that he had no experimental proof for this theory. The writer and SIDERIS of this Institute thought that they had discovered a possible function—protection against great illumination—when they accidentally found that the bodies in some plants grown in nutrient solutions, in subdued light, were much smaller than those in plants growing in full sunlight. It was found later, however, that plants grown in full sunlight in the same containers and with the same nutrient solutions also had very small silica bodies. Upon checking back it was found that the silicon supply to the plants was very low—the nutrient solutions were deficient in silica and the containers made of pyrex.

STOMATA

Stomata occur only on the underside of the pineapple leaf (13, 21, 98). According to the literature, this seems to be characteristic for Bromeliaceae in general (23, 94, 104), although several investigators have noted an exception in *Tillandsia usneoides* (110, 141, and the writer) and in a *Neumannia* species (22).

The stomata are arranged in longitudinal stripes which SOLEREDER and MEYER (141) called "stomata-bearing epidermal longitudinal stripes" or simply "stomatal stripes." These occupy the bottom and sides of the furrows already described and alternate with longitudinal stripes free from stomata located on the top of the ridges, over the vascular bundles. These alternating stripes are prominent even with low power under the microscope (13). Within the stomatal

stripe the individual stomata are not arranged in regular rows, nor are they equally spaced. The long axis of the stomatal opening lies parallel to the long axis of the leaf. The pair of guard cells exhibits an almost circular outline (fig. 150), with a diameter of 25 μ in a fully developed leaf. SOLEREDER and MEYER (141) reported the stomata of *A. comosus* as the smallest (24 μ) in all the species of Bromeliaceae they measured. In mature seedling leaves the shape of the guard cell pair is usually elliptical (fig. 142) and the diameters less.

Counts made over a period of several years on the central portion of fully developed leaves showed an average of seventy to eighty-five stomata per square millimeter. The number varies within the range given, depending upon the proportion of leaf surface occupied by ridges and furrows in each field examined under the microscope. BERGMAN and WELLER (13) reported an average of seventy stomata per square millimeter of surface.

In the strictest sense of the word, stoma (pl. stomata) includes those specialized epidermal cells, the guard cells, and the orifice or pore which they surround (30, 57). Some authors (60, 144, 146) used the word "stoma" to designate the pore only and named the combination of the pore and guard cells the "stomatal apparatus." EAMES and MACDANIELS (33) applied the word "stoma" to the opening only but added that the term might better be applied to the aperture plus the surrounding guard cells. HABERLANDT (57) went even further and suggested that the term "stomatal apparatus" should be extended to include the cells which border upon the stomatal (air) chamber, when these exhibit peculiarities in form or structure. In this paper the terms "stoma" and "stomatal apparatus" will be used interchangeably in the broader sense designated by HABERLANDT.

It is surprising that, despite the singular structure of the stomata, several investigators who have studied the structure of leaves of bromeliaceous plants have not mentioned the stomata (167), have dismissed them as having a structure similar to those on leaves of other plants (110), or have presented very brief descriptions of a few features (22, 23, 94, 141). Much the same situation exists in regard to the stomata of pineapple. WILCOX and KELLEY (166) reported the peculiar absence of stomata; BERGMAN and WELLER (13) and CARPENTER (21) found stomata but wrote simply that they were of the "usual form."

LINSBAUER (88), however, found that the stomata of the majority of the Bromeliaceae follow the same structural pattern, except for a few minor details, and that this structure is so characteristic that one can actually speak of a bromeliaceous type of stoma.

The fundamental structure can best be studied in the leaves of pineapple seedlings where the absence of thickened walls and silica bodies in the accessory cells simplifies examination. MILES THOMAS and HOLMES (98) have previously described the stomata in pineapple seedling leaves, but, since they failed to mention several features of interest, a more detailed description will be presented here. The modifications in structure which come with maturation of the leaf will also be described.

ONTOGENY.—The various stages in stomatal formation are seen to best advantage in the basal portion of a center (young) leaf from the leafy terminus of the axis of the fruiting plant (the "crown" or "top"), or on the leaf of a polyploid seedling. Since all stages in the development of the bromeliaceous stoma

have appeared in no other publication, these will be given here in detail.

The earliest stages of stomatal formation are found in that portion of the base of a young or growing leaf in which furrowing of the underside of the leaf is first evident. As seen in surface view, the formation of ridges and furrows is usually preceded by the appearance of alternating stripes of cells which are elongated in the direction of, and transversely to, the leaf axis, respectively. In the latter stripes, which will form the furrows, the stomata are initiated. This region is slightly acroscopic to that in which the first stage of trichome formation is observed (fig. 155), although some trichomes are still being formed in the region of stomatal initiation (figs. 148, 149).

DEBARY (30) outlined various types of development of a stoma from the initial cell for plants in general. He did not mention any species of Bromeliaceae as examples for any of these types, but it is obvious that the pineapple belongs to DEBARY's *2a* type. In this type the initial cell is the direct mother cell of the stoma; soon after it is marked off, a narrow piece of each of the four neighboring epidermal cells—one on each end and flank—is cut off along each of its sides by walls running nearly parallel to its walls.

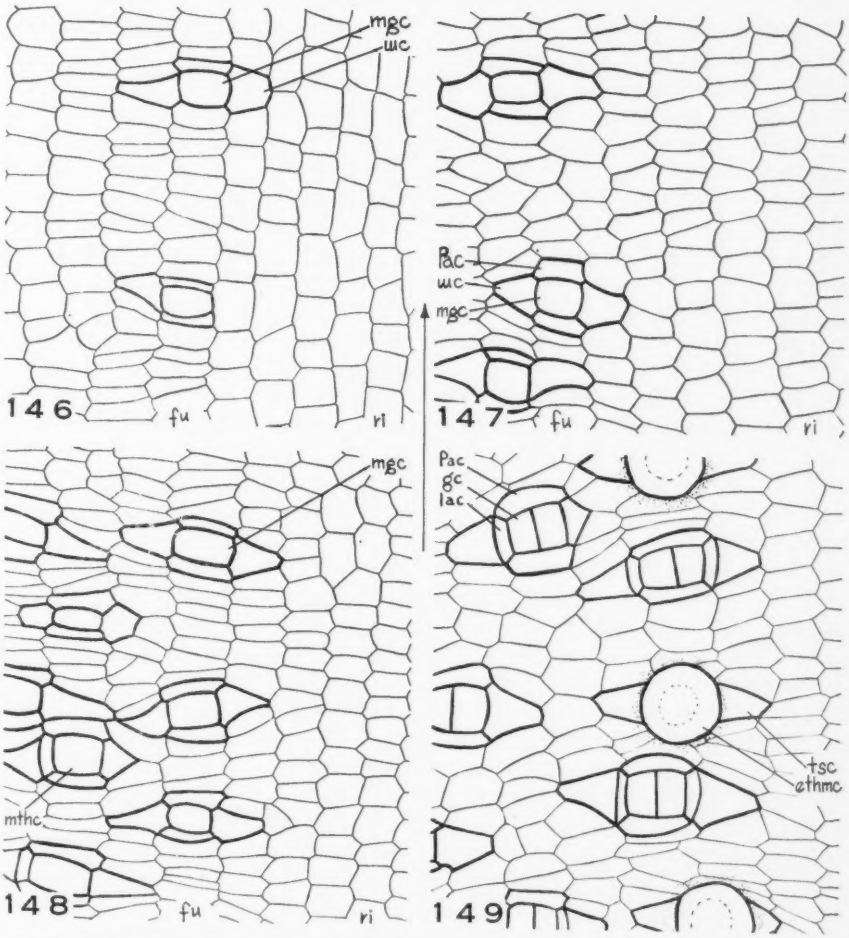
The first stage of stomatal formation is the division of a meristematic epidermal cell to form two dissimilar daughter cells, the one the initial or mother cell of the stoma and the other an ordinary epidermal cell. According to DEBARY (30), in the cases where the dermatogen cells (meristematic epidermal cells) form rows (as in pineapple), it is as a rule always the apical part of the original cell which becomes the initial or mother cell of the stoma; this is true in the pineapple leaf (figs. 142, 143). The plane wall which cuts off the initial cell is perpendicular to the outer wall and stretches transversely between and at right angles to the longitudinal lateral walls of the developing epidermal cell.

In the following description it should be remembered, when identifying the position of the cells contiguous to the stoma mother cell, that, although the mother cell is so oriented that its longest axis lies at right angles to the axis of the leaf, the positions stated here are in relation to the future guard cells, which are oriented with their longest axis in the direction of the leaf axis (figs. 142–144). Also, although the adjective "lateral" applies to all four side walls (longitudinal and transverse) of the usual cell, in a description of the stomatal apparatus this adjective is applied only to the longitudinal lateral walls or to the position of those cells bordering on the flanks of the guard cells.

Once the stoma mother cell is formed, each of the two epidermal cells lateral to it is cut by an oblique wall perpendicular to the leaf surface. The appearance of the wedge-shaped cells thus formed in each lateral position facilitates the location of the earlier stages of stomatal formation (fig. 146). In the next stage, which may occur simultaneously with, or very rapidly after, the stage just described, a narrow piece of each of the contiguous cells bordering on the ends of the mother cell (polar position) is cut off by a wall running nearly parallel to the ends, i.e., parallel to the long axis of the mother cell (figs. 146, 147), and perpendicular to the leaf surface. These two narrow cells, one at either end of the mother cell, form the polar accessory cells. Next, a narrow piece of each of the contiguous wedge-shaped cells lateral to the mother cell is cut off by a wall running nearly parallel to the sides, i.e., the

short axis in a surface view, of the mother cell (fig. 149) and perpendicular to the leaf surface. These two narrow cells, one at either side of the mother cell, form the lateral accessory cells.

The term "accessory" cells as used by recent authors (**33, 60**) is employed in this paper rather than the older term "subsidiary" cells used by earlier investigators (**30, 57, 146**). The word "subsidi-



FIGS. 146–149.—Early stomata and trichome formation. Surface views of mother cells of stomatal guard and trichome head cells, with accompanying accessory and subsidiary cells, respectively, heavily outlined to facilitate location. Fig. 146, lower epidermis from basal region of leaf from tetraploid seedling. Figs. 147, 148, lower epidermis from basal region of young prechlorophyllous leaf from leafy terminus of axis ("crown" or "top"). Fig. 149, lower epidermis from basal region of young center leaf of 8-month-old plant. *mgc*, mother guard cell; *wc*, wedge-shaped cell in lateral position to mother cell of guard cells; region of furrow, *fu*, and ridge, *ri; lac*, lateral accessory cell; *mthc*, mother cell of trichome head; *pac*, polar accessory cell; *ethmc*, expanded mother cell of trichome head; *tsc*, trichome subsidiary cell. Arrow indicates direction of leaf axis. Fig. 146, ×300; figs. 147–149, ×650.

ary" has a connotation decidedly objectionable in relation to the pineapple stomata, since the lateral accessory cells especially play a more important role in the opening and closing of the guard cells than the guard cells themselves.

After both sets of accessory cells have formed, the mother cell divides into halves to form the two guard cells. In the elongated organs of plants in general the stomatal apertures run parallel to the longitudinal axis of the organ, with few exceptions. DEBARY (30) found that in those leaves where the exceptions exist—where the apertures are at right angles to the main axis of the organ—the epidermal cells also have their longest axes at right angles to the main axis of the organ. In the pineapple leaf, however, the division wall forms parallel to the leaf axis and perpendicular to the leaf surface.

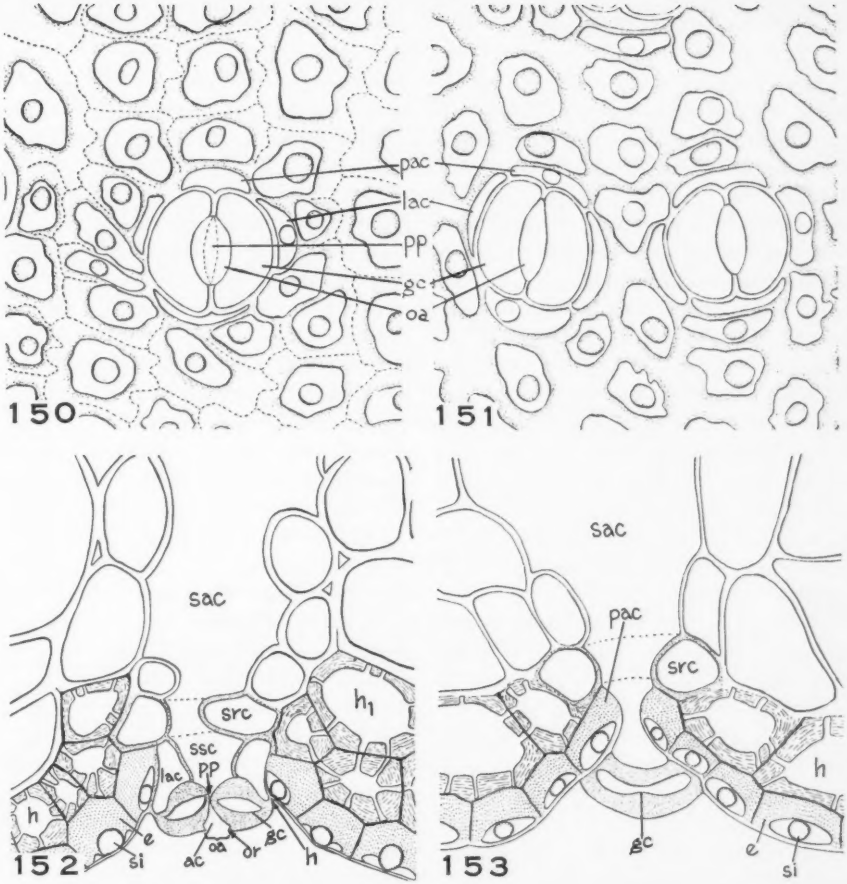
The wall which divides the mother cell then splits, in its central part, into two lamellae which gradually separate from each other. This separation proceeds from the middle toward the ends and from the (outer) aperture and the inner limit of the pore passageway to the central portion of the latter. The free edges of the outer ridge correspond to the outer edge of the original wall of division (for location of various stomatal parts mentioned in this and following paragraphs see figs. 152, 153).

Just previous to or at about the same time that the mother cell divides to form the guard cells, the substomatal chamber appears. This intercellular space originates schizogenously between the subepidermal cells. Two, or, not frequently, three, cells of the first subepidermal layer which border on the substomatal chamber are modified to form (together) a ring (fig. 145*D*, *E*).

The mother cell; the products of its division, the guard cells; and the accessory cells are of equal height and lie in the same plane as the other epidermal cells at this early stage of development. Subsequently, however, various unevennesses of height and position of these several groups of cells develop. The modifications brought about with maturation will be described later.

A surface view of the stomatal apparatus at the stage subsequent to the formation of all its units but before the thickening of cell walls presents the following picture. Each of the two guard cells, directly connected at their ends, are curved into a semilunar or sausage shape around the elliptic aperture (figs. 144, 145). This aperture represents the opening of the outer ridge and not that into the pore passageway; by focusing at a little lower level, the latter opening is usually visible as an opening lying within the former (fig. 150). Even in a surface view it is evident that the guard cells are pushed above the surface of the epidermis (fig. 145*B*). At the level of the guard cells the polar and lateral accessory cells appear as narrow cells at each end and flanking the guard cells (fig. 145*A*), but, by focusing to a deeper level, it may be seen that both the polar and the lateral pair, especially, of accessory cells arch somewhat under the guard cells (fig. 145*A*, *C*).

Focusing still deeper, one observes the peculiar substomatal ring composed of two or three cells of the first subepidermal layer (figs. 145*D*, *E*)—in a more mature region of the leaf this will be part of the first hypodermal layer. The substomatal ring bounds the lower portion of the substomatal chamber. In seedling leaves the cells forming the ring contain chloroplasts (fig. 145*D*), in contrast to the guard cells, the accessory cells, and the remaining epidermal and first layer



FIGS. 150–153.—Stomata. Figs. 150, 151, surface views of lower epidermis in chlorophyllous and subchlorophyllous regions, respectively, of mature leaf. Note irregular shape of epidermal cells surrounding guard cells, in fig. 150, especially, and thick walls and middle lamella (indicated by broken line) of epidermal cells. Broken line shown within outer aperture (*oa*) between guard cells (*gc*) indicates opening into pore passageway (*pp*), in fig. 150. Fig. 152, transverse section of lower side of fully developed leaf in subchlorophyllous region to show vertical view of stomatal apparatus. Fig. 153, vertical longitudinal section of lower side of fully developed leaf in chlorophyllous region to show different vertical view (at right angles to view in fig. 152) of stomatal apparatus. *sac*, secondary aerating canal; *src*, substomatal ring cell; *ssc*, substomatal chamber (also called "respiratory cavity" [**30**]); *pp*, "pore passageway" (**30**), also called "fissure passageway" (**57**), "stomatal opening" (**60**), "pore" (**146**), and "central fissure" by several other authors; *lac*, lateral accessory cell; *gc*, guard cell; *or*, "outer ridge" (**60**), also called "ridge of entry" (**30**); *oa*, outer aperture (also called "entrance" [**30**]; "stomatal opening" [**33**]; "pore", "outer pore", and "entrance" [**67**]); *ac*, "anterior chamber" (**146**), also called "front cavity" (**30**); *e*, epidermal cell; *si*, silica body; *h*, cell of first, and h_1, of second hypodermal layers; *pac*, polar accessory cell. ×650. Terminology used by present writer represents selection from most appropriate in previous literature and translations of terms previously not appearing in English.

of subepidermal cells, in all of which they are wanting.

In a transverse section of a leaf, showing a median vertical view of the stomatal apparatus at this same stage of development, one is able to see to better advantage the projecting guard cells and the slightly underarching accessory cells. The outer ridge and anterior chamber are also visible in this view (fig. 152). An inner ridge and posterior chamber are lacking in the stomatal apparatus in the pineapple leaf. Several authors (**22, 30, 88**) have considered this lack of an inner ridge as characteristic for Bromeliaceae.

The substomatal ring appears, in a transverse section, as two isodiametric cells beneath the lateral accessory cells. By focusing to a greater depth, one perceives that the "two cells" are parts of a ringlike structure, lying in the same plane (fig. 152). There is no such modification of these ring cells whereby the ends are bent inwardly, at the same time curving upward to project up in the vicinity of the guard cells, as LINSBAUER (**88**) reported for many other bromeliaceous species.

Further development of the stomatal apparatus consists chiefly of the modifications brought about with further growth and thickening of the walls. The guard cells are pushed even more outward by the lateral accessory cells which in turn are pushed beneath the former. The walls of the ordinary epidermal cells, of the guard cells, of the polar accessory cells, and of the hypodermal cells, with the exception of those bordering the substomatal chamber, all become greatly thickened. Only the last-named cells and the lateral accessory cells remain comparatively thin-walled. The functional significance of the thinner walls will be described later.

MATURE STRUCTURE.—The appearance of the mature stomatal apparatus, seen best in a median vertical view in a transverse section of the leaf (fig. 152), is as follows. The guard cells, ovoid in cross section, have very greatly thickened walls, the lumen as a result appearing hardly more than a slit. The lumen is usually oriented somewhat obliquely inward, i.e., toward the pore passageway, and downward. Not visible in this view but in a longitudinal section of the leaf cut through a stoma (fig. 153), it may be seen that the lumen widens out toward the ends (polarly) where the guard cell wall is not so thick; according to LINSBAUER (**88**), this is characteristic for bromeliaceous guard cells. There is no true back wall in the guard cells. According to LINSBAUER, this condition is characteristic for Bromeliaceae except in a few cases where the guard cells are approximately quadrilateral in cross section. There is a definite if not very pronounced outer ridge with an anterior chamber between the (outer) aperture and the opening into the pore passageway. In vertical section the anterior chamber is funnel-shaped. The pore passageway is extremely short and opens abruptly into the substomatal chamber, since an inner ridge and posterior chamber are lacking.

Lying almost completely under the guard cells are the lateral accessory cells. The inner and lateral walls, facing the substomatal chamber, of each of these latter cells are thin. The outer wall of the cell is reduced to a narrow strip and is also thin. This latter wall serves as a hinge. Its hingelike structure can best be seen in a vertical view of the stomatal apparatus in a transverse leaf section. It is by means of this hinge that the guard cell is mobile. This will be discussed later in a description of the functional processes of the stomatal appara-

tus. HABERLANDT (**56**) considered the inner wall also a hinge, naming it the "inner hinge," but in the pineapple, at least, this inner wall does not serve as a hinge, and the term does not apply. The lumen of these lateral accessory cells narrows toward the outside—in the direction of the outer wall, between the guard cell and adjacent epidermal cell. Because of the presence of chlorophyll in the lateral accessory cells of bromeliaceous species he studied, MEZ (**94**) considered these cells to be part of the mesophyll. LINSBAUER (**88**) pointed out the error of this conclusion, indicating that these cells are definitely part of the epidermis. The presence of chloroplasts he correlated with the function of the lateral accessory cells. A study of the early stages in the development of a stoma, as outlined in previous paragraphs, shows without question that these cells are originally epidermal cells and definitely part of the epidermis. They also contain silica bodies.

The polar accessory cells, with their thick lateral and inner walls, project from under the guard cells and arch toward the substomatal chamber, thus somewhat narrowing the latter (fig. 153). LINSBAUER (**88**) has written that *Ananas* represents an extreme case of this type of development, which is rare among other species of Bromeliaceae. These projecting polar accessory cells give an unusual appearance to the stoma in a surface view, since they project into the substomatal cavity beneath the guard cells, and the silica body which each contains shines out through the guard cell above. LINSBAUER (**88**), writing of Bromeliaceae in general, thought that such polar accessory cells served a mechanical function in protecting the stomatal apparatus against the tearing and pulling effects of the accordion-like movements of the water-storage tissue beneath. By causing a narrowing of the substomatal chamber, these accessory cells may also contribute toward a reduction in transpiration.

The ordinary epidermal cells surrounding the guard and accessory cells are somewhat modified during the maturation processes which the stoma cells undergo. This is seen to best advantage in a surface view. The modification is chiefly one of orientation (figs. 150, 151), the result of these adjacent cells being pushed out of alignment through the original cutting-off of the accessory cells and especially through the further development of the latter. Although in many instances the arrangement of the adjacent epidermal cells does not seem to follow any definite design (fig. 151), more often they form a ring around the guard and accessory cells (fig. 150). In this latter case, especially, and to some degree in the former case, it seems likely that these adjacent cells serve to protect the stomata from longitudinal stresses of the leaf, a function attributed to somewhat similar but more specialized "rings" described by MEZ (**94**) and LINSBAUER (**88**) in other Bromeliaceae. LINSBAUER described these as "accessory" cells, but even if the epidermal cells surrounding the stomatal apparatus serve the mechanical function suggested, they can hardly be considered "accessory" cells, since they serve no purpose in the actual "operation" of the stomatal apparatus. They will therefore be considered in this paper as modified epidermal cells surrounding the guard and accessory cells.

Since the surface of the cells just described is below that of those ordinary epidermal cells adjacent to them, a stoma appears to lie in a slight depression in the furrow in which the stomatal stripe is found. These depressions in

which pineapple stomata lie cannot actually be called "crypts," as they have been for other bromeliaceous species (88, 94, 141).

Beneath the accessory cells, limiting the substomatal chamber inwardly, is the ring of thin-walled hypodermal cells. The aperture formed between the ring cells opens into a secondary aerating canal, which has already been described.

FUNCTIONING OF STOMATAL APPARATUS.—The opening and closing of the pore passageway in the stoma of the pineapple does not depend upon the independent movement of the guard cells themselves, as in the stoma of most plants, but indirectly upon that of the lateral accessory cells, primarily, and upon that of the substomatal ring, secondarily.

MEZ (94) has already described the manner in which the bromeliaceous stoma functions, regardless of the variation in structural details found in the different genera. Essentially, this is as follows for the pineapple stoma. The great thickening of the walls of the guard cells and the absence of chloroplasts in these cells preclude their movement through a gain and loss in turgor as in other plants, so this role of movement is taken over by the thin-walled lateral accessory cells. Since these cells lie beneath the guard cells, they push up on the guard cells when turgid and force them apart. When not turgid, the front walls of these accessory cells, facing the substomatal chamber, collapse, resulting in a downward and closing movement of the guard cells. This passive movement of the guard cells is facilitated by the presence of the hinge. Loss and gain in turgor of the thin-walled, chloroplast-containing cells of the substomatal ring, adjacent to the lateral accessory cells, secondarily influences the movement of the guard cells. The polar accessory cells play no part in the movement of the guard cells but, as noted previously, probably contribute to a diminution of transpiration by their partial obstruction of the substomatal chamber.

The scope of this investigation did not permit a study of the functional period of stomata in pineapple, but indications are that they are nonfunctional in the older portions of a fully developed leaf. MEZ (94), describing Bromeliaceae in general, believed that their stomata soon cease to function, except in some species which inhabit damp regions. BILLINGS' (14) inability to demonstrate experimentally any movement in the guard cells of *Tillandsia usneoides* may be due to his failure to understand the mechanism of bromeliaceous stomata.

PELTATE SCUTIFORM TRICHOMES

TERMINOLOGY.—The terminology used for the individual members constituting the hairy covering characteristic of the leaves of bromeliaceous plants has been various since their presence was first noted by RUDOLPHI (113). He and many others (6, 11, 14, 19, 30, 34, 61, 72, 82, 95, 99, 100, 106, 110, 116, 117, 150, 167) simply called it a "scale," from the scalelike form of the trichome head. Without reference to the type of hair as indicated by the earlier name "scale," other authors (22, 23, 32, 88, 95, 109, 126, 148, 150, 153, 156) merely termed the structure a "hair" or used the technical equivalent "trichome." Writers later combined the two terms to make "scale hair" or "scalelike trichome" (6, 94, 95, 118, 120, 142, 150, 167) or the equivalent "shield hair" (7, 141). The pedicellate nature of the trichome was indicated by the more recent names of "peltate hair" (7) or, more specifically, "pedicellate scales" (12, 13, 72). The functional

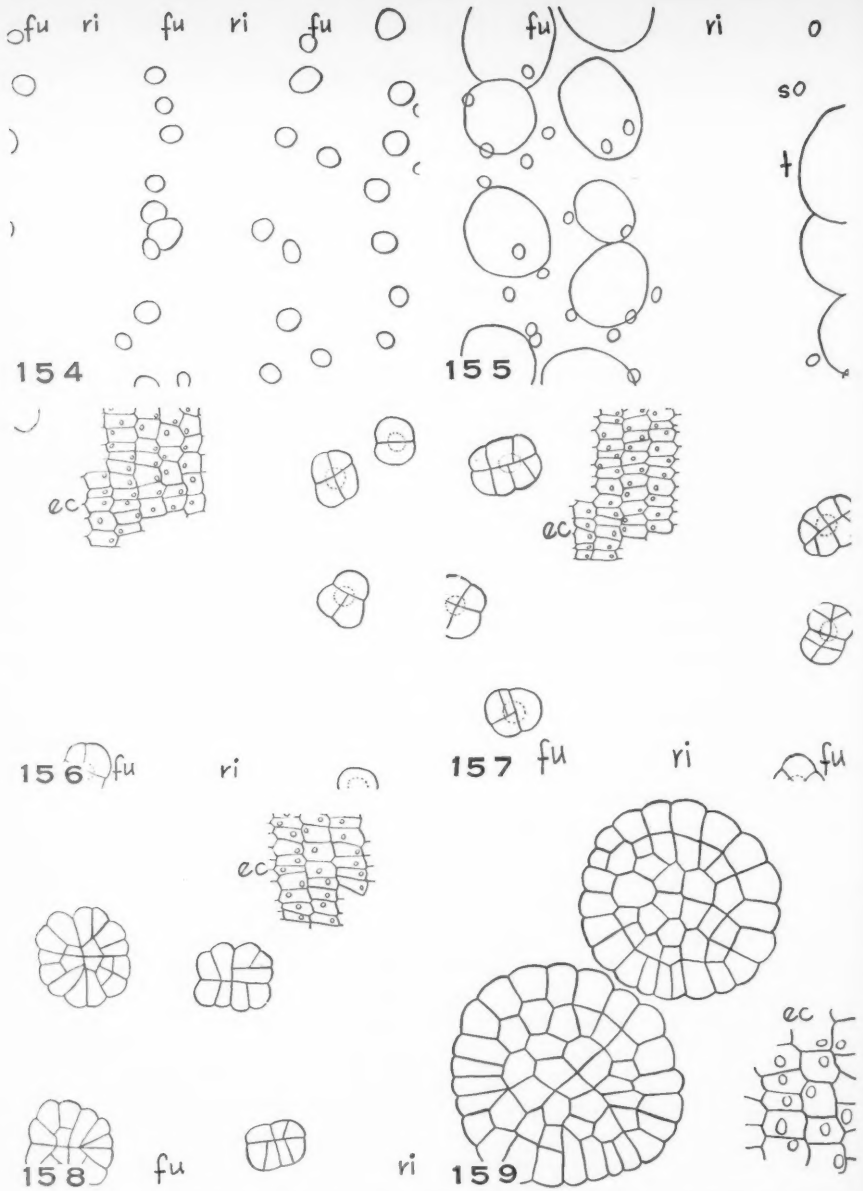
designations of "(water) absorbing hair," "scale," or "trichome" (**57, 107, 131, 143, 144, 160**), and "glandular hair" (**98**) also appear in the literature; the last term is used erroneously, since the bromeliaceous trichome is in no way glandular in the true sense of the word. DEBARY (**30**) used the term "peltate scutiform trichome," which in nontechnical language is a shieldlike hair attached by its lower surface to a stalk. Since this seems the most descriptive and correct, it will be used by the writer.

The scutiform trichome is uniquely characteristic for the Bromeliaceae (**167**) and is found on the leaves of most members of this family (**22, 110**), with few exceptions (**22, 94, 141, 150**) where stellate and fasciculate trichomes are variants of the more common type.

REVIEW OF LITERATURE.—A brief review, in chronological order, of the literature on the bromeliaceous trichome will give some idea of the interest aroused by this structure, chiefly because of its function of absorbing water, especially in epiphytic forms. For a long time, according to RUDOLPHI (**113**) and CEDERVALL (**22**), the covering of trichomes was thought to be a disintegrated portion of the epidermis. RUDOLPHI, EHRENBERG (**34**), SCHACHT (**116, 117**), and VON MOHL (**100**) are the earliest anatomists known to have described the occurrence of scalelike trichomes on bromeliaceous plants. VON MOHL made tests to determine the chemical nature of the scales.

DEBARY (**30**) described a definite hair form (the shieldlike scales) as characteristic for this family. SCHIMPER (**118, 120**), in his expositions on the epiphytic vegetation in tropical America, described the structure of the scales and first demonstrated their function of water absorption in both epiphytic and terrestrial forms. CEDERVALL (**22**) characterized the trichomes as having a second important function—protection of the leaf against excessive transpiration. Since in the pineapple, at least, the trichome heads form a rather thick mat over the stomata, it seems probable that the trichomes do play such a role.

BACHMAN (**7**), in a study of shield hairs in monocotyledons, classified them according to the shape of the shield and the arrangement and shape of the shield cells; he limited his studies of Bromeliaceae chiefly to the genus *Tillandsia*. WITTMACK (**167**) in the main cited studies of others on the structure of the trichomes of several other bromeliaceous species but also described the scales in several species he himself had investigated. RICHTER (**110**) divided bromeliaceous trichomes into two groups: those with heads lying flat on the surface of the epidermis (to which group the pineapple belongs) and those sunken below the surface. He also observed a relationship between the species habitat and the density of the trichome covering and demonstrated that the trichomes on the upper surface of leaves of various terrestrial forms of Bromeliaceae absorbed water, while those on the lower surface did not. BULITSCH (**19**) described the ontogeny and mature trichomes of *Billbergia iridifolia*. MEZ (**94**) classified trichomes of various genera and species of Bromeliaceae as belonging to either one of two general types: the one with a head consisting of a central portion with four cells, surrounded in turn by regular successive concentric layers of cells, and the other with a head having a central portion consisting of a single cell, which is surrounded by an irregular network of cells. The form most common to the pineapple is a type intermediate between MEZ's two types. WALLIN (**156**) described the presence of tannin in the



FIGS. 154–159.—Trichomes and stomata. Fig. 154, surface view of underside of leaf at base to show outlines of scutiform trichome heads. In very basal portion (below) only trichomes are present, scattered irregularly across width of leaf, and, acroscopically, more or less aligned in stripes (above). Fig. 155, surface view, in region acroscopic to one shown in fig. 154, to show outlines of (*s*) stomata (small circles) which are formed in same stripe as trichomes; heads (*t*) of latter enlarging (larger circles and ellipses). Figs. 156–159, surface views of lower epidermis of basal, nonchlorophyllous region of leaf from 6-month-old plant to show arrangement, distribution, and stage of development of trichome heads, and relative size and shape of epidermal cells in given areas of this leaf region. Fig. 156, area 2.4 mm. above base of thirtieth leaf (leaves counted from base of stem). Figs. 157–159, on thirty-first leaf. Fig. 157, 1.6 mm. above base; fig. 158, 2.4 mm. above base; fig. 159, 4 mm. above base. *ec*, epidermal cells, each containing silica body; *fu*, region in which leaf furrow develops, and *ri*, ridge; outline of stoma (*s*); of trichome head (*t*). Dotted line within trichome head boundary represents cross section of trichome stalk. Figs. 154, 155, ×200; figs. 156–159, ×300.

basal cells of the trichomes of many bromeliaceous plants.

HEDLUND (61) was the first to describe the development of the bromeliaceous trichome in general, while BILLINGS (14) studied its development on *Tillandsia usneoides*. MEZ (95) gave detailed descriptions of the structure of the trichomes of various *Tillandsia* species and the mechanics of water absorption by them, while STEINBRINCK (143) studied the function of the trichomes of bromeliaceous plants in general. TIETZE (150) presented a detailed morphological study of a great many bromeliaceous species. ASO (6) studied the absorption of dissolved salts by trichomes on various species, including *A. comosus*. LINSBAUER (88) and PICADO (106) chiefly reviewed the work of previous authors. HABERLANDT (57) described the structure and function of the trichomes, basing his data for the most part on the earlier researches of SCHIMPER and MEZ. KEILINE (72) found that the trichomes could be divided into two distinct groups, from an anatomical point of view: the one form occurs on epiphytic, the other on terrestrial species of Bromeliaceae. The authors CHODAT and VISCHER (23) contributed certain details on the thickening of various walls. BARBAINI (11) described the structure and function of trichomes in several *Tillandsia* species. WETZEL (160) carefully investigated the cutinization of the walls of the stalk cells. STAUDERMANN (142) studied the relationship between the trichome form and "topographical position," i.e., their location on the leaf; classified such trichomes according to certain anatomical features; and studied their functional relationships with certain environmental factors. SOLEREDER and MEYER (141) recently contributed an extensive summary of their own researches and those of some of the previously quoted authors on the form of trichomes in the Bromeliaceae.

Some of the above-mentioned authors (6, 141, 150, 167) incidentally have given brief descriptions and/or illustrations of the trichomes of the genus *Ananas*, including *A. comosus;* others (13, 68, 131) have also presented short descriptions of the trichomes on leaves of mature pineapple plants, and MILES THOMAS and HOLMES (98) of those on seedling leaves.

The silvery-white furfuraceous covering, which is such a striking feature of the lower surface of the more mature and exposed portions of the pineapple leaf, consists of the air-filled scalelike or shieldlike heads of the trichomes (fig. 172). Chiefly because the trichomes on the upper surface are fewer in number and because they lie flatter against the surface, the upper surface appears glabrous to the unaided eye in contrast to the "scurfy" lower surface. Microscopic examination of the trichomes on the two surfaces of the leaf shows a slight difference in form also; this will be described later.

On the lower surface of the leaf the bases of the trichomes are arranged in longitudinal stripes, located only in the furrows—on the bottom of the furrows and to some extent on the sides or slopes of the ridges but never on top of the ridges. On the upper surface of the leaf, which is not ridged, the bases of the trichomes are also arranged in more or less regularly spaced longitudinal stripes which alternate with stripes free from trichome bases. Within the furrow on the lower, and in the stripes on the upper, side of the leaf, the trichome bases are not arranged in any regular order. Counts made over a period of several years on the central portion of fully developed leaves showed an average of twenty-five to thirty trichome bases per square millimeter on the lower side and

about half that number on the upper side. Other writers (**13, 110**) have previously commented on the more abundant occurrence of trichomes on the lower than on the upper surface of bromeliaceous leaves, including *A. comosus*.

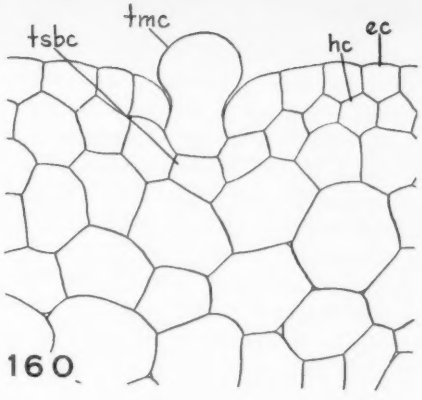
ONTOGENY.—The earliest stages of trichome development can be observed, in the basal regions of young prechlorophyllous and older growing leaves. They are first visible a short distance from the base of the leaf on the lower side in which ridges and furrows, characteristic for this side, have not yet formed. In this lowermost area of trichome formation they are thinly scattered without any noticeable arrangement (lower part of fig. 154). The shape of these trichomes, when mature, often differs from that of the great mass found on the leaf.

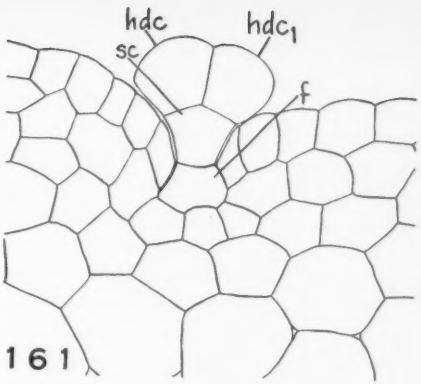
Just acroscopic to this narrow zone, developing trichomes are found aligned into longitudinal rows, alternating with stripes of cells in which no trichomes form (figs. 155–157, 170). The "trichome stripes," which coincide with the "stomatal stripes" already described, and the stripes without trichomes and stomata predesignate the later-formed furrows and ridges, respectively. The trichomes begin to form in a region slightly basiscopic to that in which stomata are initiated, i.e., in more meristematic tissue (fig. 154). On the upper surface of the leaf the trichomes begin to form in a zone several centimeters farther from the leaf base than do those on the lower surface.

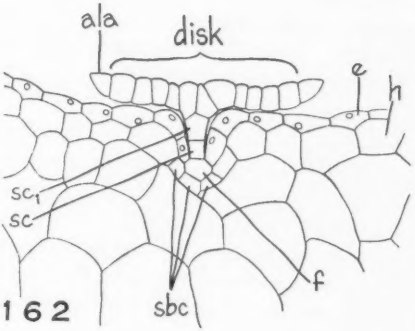
A surface view of such a meristematic region, with the microscope focused at the level of the epidermal cells, shows that the formation of the mother and the subsidiary cells of a trichome is closely similar to that of a stoma and its accessory cells (fig. 148). Upon focusing to a level a little above the epidermal cells, however, one sees what appears to be a clear spherical body lying upon the epidermis immediately above the mother cell (figs. 149, 167*A*). Examination of transverse sections made of a leaf in this region shows that this bubble- or blister-like structure is an expanded portion of the trichome mother cell (fig. 160). Another feature which helps distinguish the trichome from the stoma at this very early stage of development is that the mother cell of the former becomes deeply colored by haematoxylin stains. HEDLUND (**61**) observed this, also, in *Karatas plumieri*.

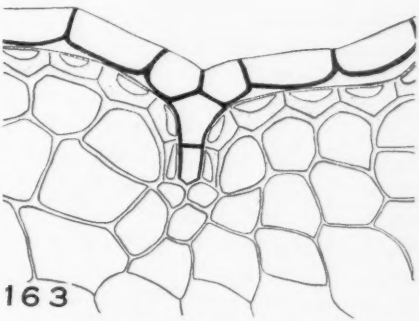
The sequence in formation of the subsidiary cells is similar to that of the stomatal accessory cells. Although the epidermal cells immediately adjoining the trichome base are modified and take a secondary part in the function of the trichome, they do not play the important role that those surrounding the stoma do, and they should, therefore, be called subsidiary cells. These cells, four in number, will form a ring around the base of the stalk of the trichome (fig. 165).

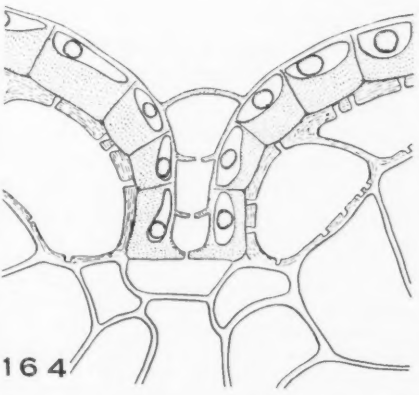
The trichome mother cell begins to expand above the surface of the subsidiary cells immediately upon the cutting-off of the latter cells, or even during the final stages of the latter process, to form the hemispherical structure already mentioned. Once the mother cell is differentiated, the subsidiary cells, at first, and the surrounding ordinary epidermal cells, for a longer period of time, grow at a much more rapid rate than the trichome mother cell so that the basal portion of the expanding trichome mother cell lies sunken in a depression (fig. 160). In the next stage of development a transverse (periclinal) wall forms in this basal portion of the mother cell at the level of the outer wall of the adjacent subsidiary cells (fig. 161). The basiscopic cell thus produced undergoes no further division. It is in a

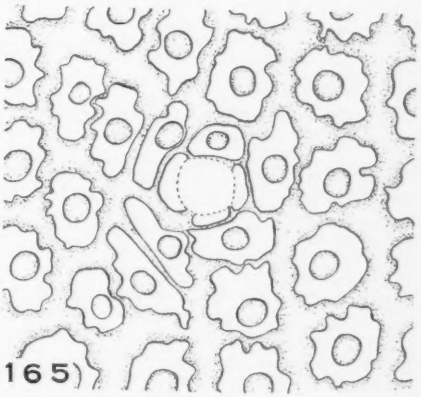












FIGS. 160–165.—Trichomes. Figs. 160–163, transverse sections of lower sides of leaves to show vertical views of successive stages of trichome development. Fig. 160, first stage (in young, prechlorophyllous leaf from leafy terminus of axis—"crown," or "top"): expansion of trichome mother cell (*tmc*), formed from epidermal cell (*ec*). *tsbc*, small cell of first hypodermal layer, *hc*, at base of (future) trichome stalk. Second

[Legend continued on following page]

line continuous with the other cells of the epidermis and forms the lowermost or basal cell of the stalk of the trichome. This latter cell is distinguishable from the later-formed cells of the stalk primarily by its position. As a result of its position, however, during early development it is subjected to certain pressures from adjoining cells which cause it to have a somewhat different shape from the other cells of the stalk (figs. 161, 162). In the acroscopic cell of the two produced by the first division of the mother cell, two transverse (periclinal) walls form in succession, so that finally the completed stalk consists of a single vertical row of three cells (fig. 162). The basal cell is now somewhat larger than the other two stalk cells. From this stage on the basal stalk cell and adjacent subsidiary cells grow very little, whereas the stalk cell beyond the basal cell increases in height considerably; and the diameter of the acroscopic stalk cell increases without a corresponding increase in height, so that a broader cell is formed. This acroscopic cell of the stalk is called the "cupola." Thus the stalk conforms in shape to the funnel-shaped depression which it now fills (fig. 162).

We must go back again to an earlier stage in development in order to follow the formation of the trichome head. When the vertical row of (stalk) cells is being formed, the outermost of these cells which was cut off when the last periclinal wall develops, and which bulges somewhat at the end of the stalk, is the initial cell of the trichome head, or "scale," itself. This cell divides in half into two daughter cells, through an anticlinal wall (figs. 156, 161, 167*B*). In some cases the acroscopic cell of the stalk is formed after, instead of before, the initial cell of the head divides (fig. 161). Soon after the two daughter cells are formed, the head is divided into four cells by anticlinal walls at right angles to the first (figs. 156, 167*C*). These original four daughter cells then increase in size and divide rapidly by a series of anticlinal walls at right angles or oblique to the first-formed anticlinal walls (figs. 157, 158, 167*D*, *E*). The identity of the original four daughter cells is thus lost. Consequently, the distinct central group of four cells so characteristic for *Tillandsia* trichomes (and assumed by many authors to be characteristic for all members of the Bromeliaceae) is seen but rarely in the mature pineapple trichome head (figs. 169, 178).

Growth of these first-formed head cells and their division by radial and tangential (anticlinal) walls—in relation to the periphery of the structure as a whole—result in a single layer of cells in the shape of a disklike or shieldlike (scutiform) "head." Since the division of cells follows no regular order, the pattern of cell ar-

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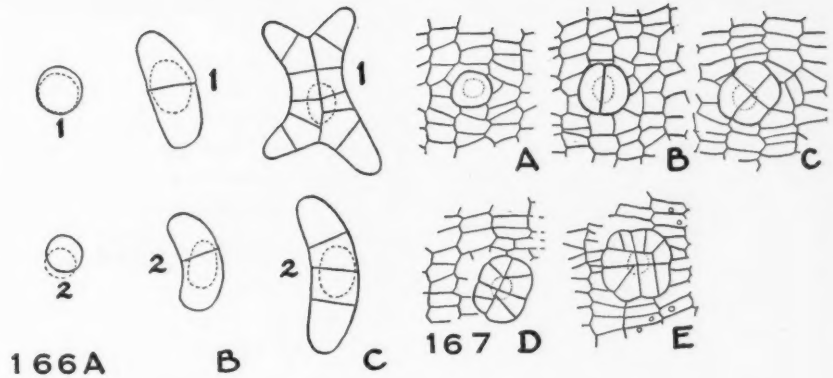
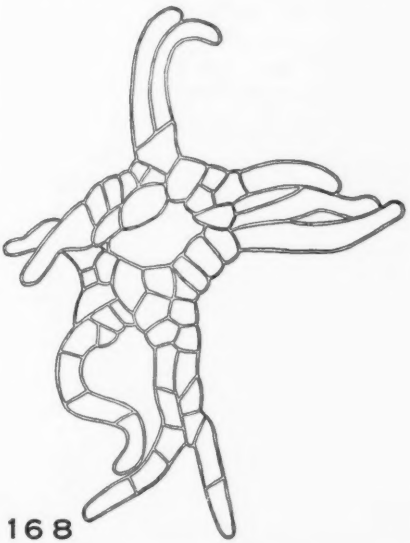
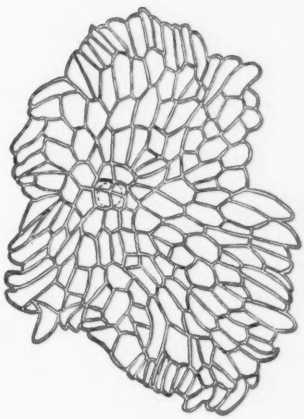
stage (not illustrated): formation of first periclinal wall to cut off basiscopic cell (foot, *f*, in fig. 161) of trichome stalk. Fig. 161, third and fourth stages (in same leaf as for fig. 160): formation of second periclinal wall to make second stalk cell (*sc*), and initial mother cell of trichome head (latter stage not shown). Trichome head mother cell divides anticlinally to form first two daughter cells of head, *hdc* and hdc_1, Fig. 162, later stage in seedling leaf, where trichome head is considerably (but not fully) expanded to form disk and ala; and stalk is complete with basal cell, the foot (*f*), center cell (*sc*), and acroscopical cell or cupola (sc_1). *sbc*, modified hypodermal cells around base of stalk foot; *e*, epidermis, *h*, hypodermal cell. Fig. 163, stage (in fully developed leaf) found on mature portions of leaf, with inner and lateral walls of trichome head thick and outer walls collapsed and lying against inner and lateral walls. Walls of stalk cells and adjacent epidermal and hypodermal cells also thickened. Figs. 164–165, basal portions of trichomes on mature leaves to show loss of head as result of "weathering," and thickening of walls. Fig. 164, vertical longitudinal, and fig. 165, surface views. Figs. 160–161, 164–165, ×650; figs. 162–163, ×300.







FIGS. 166–169.—Trichomes. Fig. 166, successive early stages of development of two trichome heads (series *A1–C1*, and *A2–C2*) of the irregularly shaped, scattered (isolated) type (figs. 168, 177) from upper surface of basal portion of young leaf from 6-month-old plant. Stages of trichome heads illustrated here occurred at following distances from base of leaf: *A1, 2*—0.2 mm; *B1, 2*—0.4 mm; *C1*—0.2 mm. *C2*—0.8 mm. All trichomes may not be at same stage of development at same distance from leaf base, but this is usually more or less true. Note that central cell of trichome head in fig. 168 is undivided; not the usual case. This type of trichome similar to those found in nodal region of stem. Fig. 167*A–E*, surface views of selected portions of upper epidermis at upper limit of basal, nonchlorophyllous region of young leaf from 6-month-old plant to show successive early stages of development of trichome head of more usual scutiform type shown in fig. 169. Broken line indicates cross section of trichome stalk. Trichome heads illustrated here occurred at following distances from base of leaf: *A*, 1.2 mm; *B*, 1.4 mm; *C*, 1.8 mm; *D*, 6.6 mm; *E*, 6.9 mm. Figs. 166–168, ×300; fig. 169, ×200.

rangement in the head is highly variable and rather irregular (fig. 169), although the cells usually seem to be arranged roughly in concentric rings (**141, 150**).

The peripheral cells of the head continue to divide until the structure has reached its mature size. The main portion of the head is known as the disk. The marginal cells finally become greatly elongated radially to form the ala (depicted later in this section).

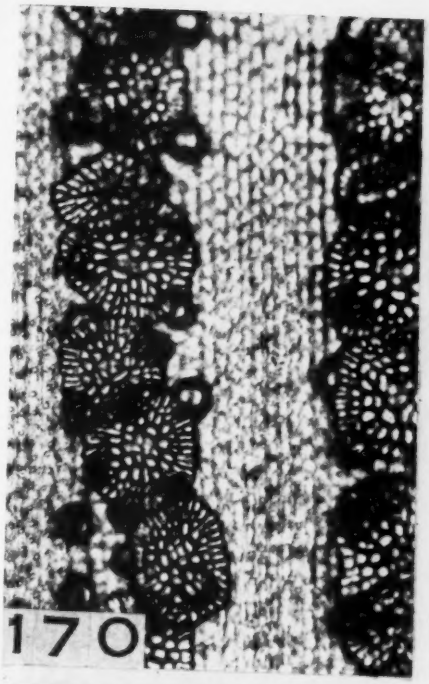
The development of the scutiform trichome on the pineapple leaf resembles in many respects the development, at least in the earlier stages, of the trichomes in *Billbergia iridifolia* (**19**), *in Karatas plumieri* (**61**), and in *Tillandsia usneoides* (**14**).

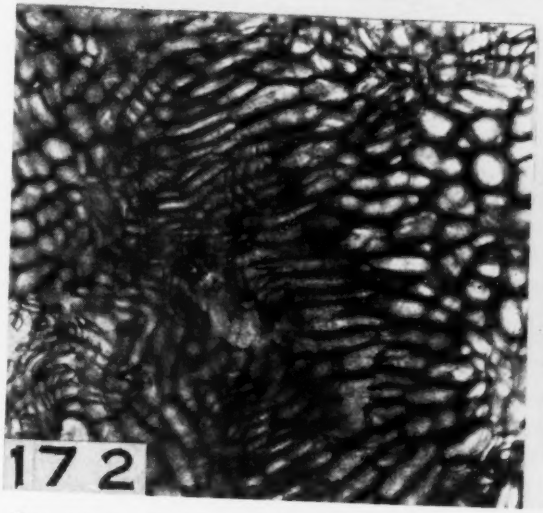
VARIANT FORM.—A variation of the scutiform head is the much more irregular (in outline) stelliform head. In the latter's development the stalk and mother cell of the head are formed in the same manner as in the major type. Just after the first anticlinal division of the mother cell of the head, the two daughter cells elongate in such a manner as to produce together, as seen in surface view, a subelliptical (fig. 166*B1*, *2*) rather than the subcircular shape described for the scutiform type. The next two anticlinal division walls do not form at right angles to the first, as in the scutiform type, but parallel to it, or nearly so. Thus a single row of four cells (fig. 166*C2*) rather than four wedge-shaped ones results. Subsequent division and growth of these original cells are much more irregular than in the first type (fig. 166*C1*), resulting in a starlike rather than a disklike structure (figs. 168, 177). There appears to be no specific correlation between the location on the leaf and the form of the trichome head, although the stelliform type was found more often on the upper than on the lower surface and more frequently on the base than on the blade proper.

There are, therefore, two rather diverse types of trichome heads on the pineapple leaf: the scutiform, which is the form most prevalent on, and which covers the greater part of, the blade on both the upper and the lower surfaces, and the stelliform, which usually occurs as isolated trichomes at the base; there are also transition forms (figs. 174–176). Although most of the literature on the occurrence of trichomes in the Bromeliaceae gives the impression that the trichome head is quite specific for a species, and even for a genus (**13, 98, 126, 150**), several authors (**7, 94, 141, 142**) have reported a situation similar to that found in the pineapple.

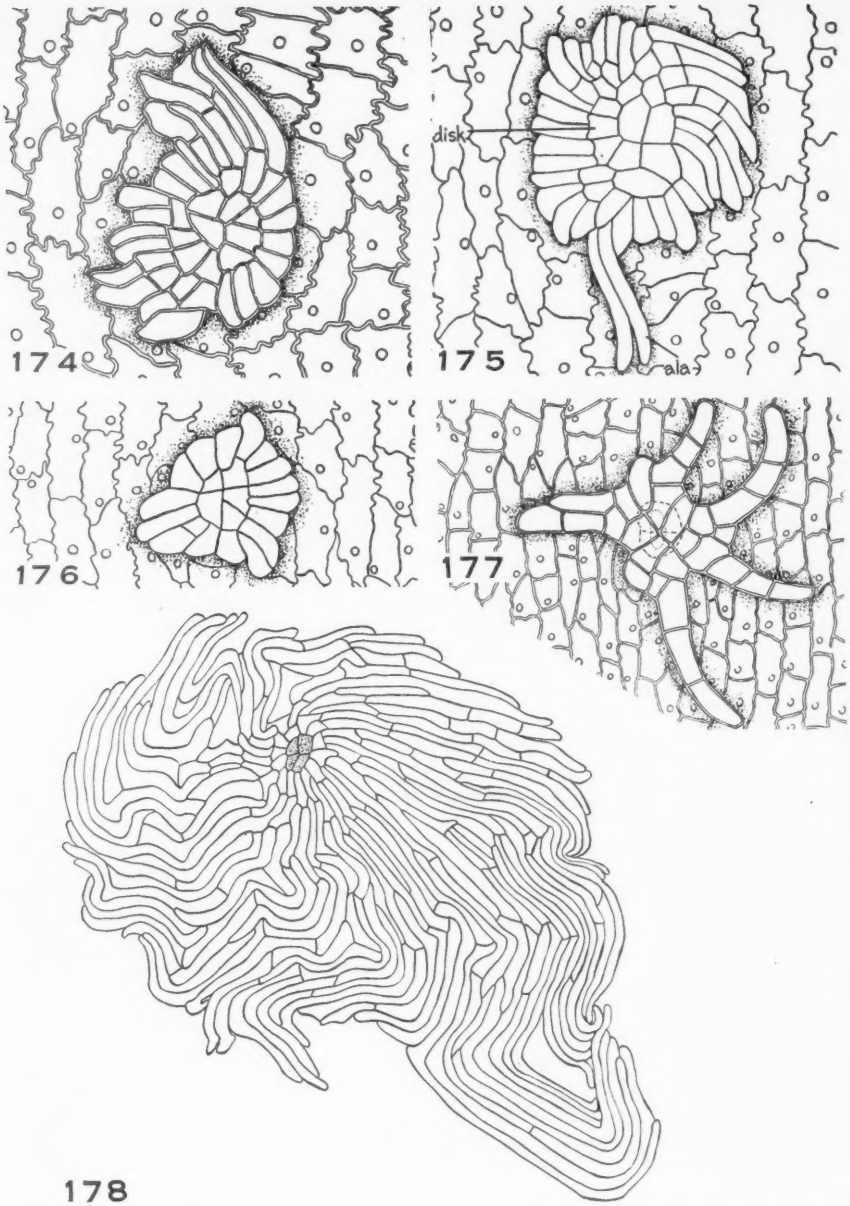
On the lower surface of the leaf, during the period when the cells in the trichome head divide and enlarge, the head margins advance up the sides of the furrows to the top of the ridges. Thus the margins of the heads of trichomes in adjoining furrows approach one another and finally overlap on the ridge (figs. 170, 171). Since the bases of the trichomes occur rather close together in the furrow itself, the margins of the heads overlap one another within the furrow as well. Thus, in a mature portion of the leaf blade the covering appears several layers thick because of the overlapping of the margins of many adjacent trichome heads (fig. 172).

MATURE STRUCTURE.—When the development of the trichome is complete, the picture presented by an individual member of this heavy, scurfy covering is as follows. On superficial examination of a transverse section of the leaf to show a median vertical view of the structure it appears as if the stalk of the trichome is "sunk beneath" the epidermis, as various





FIGS. 170–173.—Trichomes and marginal spine. Figs. 170–172, surface views of underside of fresh leaf to show: confinement of trichome heads in furrow at early stage of development (fig. 170)—small circular structures near margins of trichome heads are stomatal guard cells; advancement of enlarging trichome heads up slopes of furrows to crests of ridges (fig. 171); and final complete covering of leaf surface (fig. 172). Note overlapping of adjacent trichome heads. Darkness of cells of trichome due to air under heads (fig. 171, especially). Fig. 173, vertical longitudinal section of young (seedling) leaf to show early stage of spine development. Spine is initiated in tissue which remains meristematic at margin of leaf. Figs. 170–172, ×250; fig. 173, ×200.



FIGS. 174–178.—Trichomes. Figs. 174–177, surface views of trichome head types found scattered (isolated) on upper surface of basal, nonchlorophyllous region of leaves from 6-month-old plants. Counting green leaves only, trichomes illustrated were found on following leaves (first number) given distance from base (second number): Fig. 174, seventeenth—175 μ; fig. 175, twenty-third—0.8 mm; fig. 176, twenty-fifth—0.6 mm; fig. 177, thirty-seventh—0.8 mm. Fig. 178, surface view of trichome head on underside of basal portion of leaf. Figs. 174–177, ×300; fig. 178, ×200.

authors have written. With closer observation it is immediately seen that the stalk actually lies in a depression of the leaf surface, in addition to the trichome as a whole lying in a leaf furrow. The epidermal cells and the underlying hypodermal cells surrounding the stalk grow so much more rapidly than the basal and other cells of the trichome stalk and of the subsidiary cells that the stalk remains completely inclosed within the depression thus formed. The depression and the stalk which fits closely within it are funnel-shaped. The stalk consists of three cells aligned in a vertical row: a small basal cell; a central cell with a relatively small diameter but considerable height, in the form of a column, and constituting the stem of the funnel; and the acroscopic cell, the cupola, which forms the funnel cone (fig. 163). Surrounding the basal cell and at about the same level are the four small subsidiary cells. Comparison of a trichome at this stage of development and at the early one previously described indicates that the basal cell and the subsidiary cells increase very little in size once they are cut off, whereas the other stalk cells and surrounding epidermal and hypodermal cells enlarge considerably (figs. 162, 163). With maturity the inner and lateral walls of the epidermal cells which line the sides of the depression become greatly thickened, lignified, and finally cutinized similarly to the other epidermal cells. Eventually the lateral walls of the central and acroscopic cells of the stalk become coalescent with the outer walls of the epidermal cells lining the depression into which the stalk fits (fig. 164). The walls of the basal stalk cell and the subsidiary cells, however, remain thin and unlignified for a longer period of time; eventually they, too, become lignified.

The head consists of the disk—the main central portion—and of the ala—the peripheral portion extending beyond. The disk usually consists of a single layer of nearly globose cells, although they may be polygonal or greatly elongated in a radial direction (figs. 162, 169, 178). The central cells of the disk are usually smaller and more or less depressed in relation to the surrounding cells of the head. SOLEREDER and MEYER (141) believed this condition to be characteristic of trichomes in furrows. The cells are arranged in rather irregularly concentric rings. The ala consists of the peripheral row or rows of cells in the head and is distinguished from the disk not only by its position but by certain morphological features. The ala cells are usually greatly elongated radially, and, since the radial lateral walls of adjacent cells are noncontiguous along part of their length, the cells project finger-like from the disk. This condition has led many authors to call the ala the "lobed" portion. These digitaliform cells often are very long and narrow, becoming "bent" in various directions, resulting doubtless from the obstruction of adjacent trichome heads (fig. 178).

During the period when the cells of the trichome head are dividing and increasing in size, they as well as those of the stalk contain living protoplasm, including prominent nuclei. Soon after division and growth cease, however, the protoplasmic contents of the cells forming the head disappear, and these cells become filled with air. The protoplasmic contents of the stalk cells persist longer than in the head cells.

The inner and lateral walls of the disk portion of the head become greatly thickened, while their outer walls and all walls of the cells comprising the ala remain thin. Eventually, the outer walls of the cells in the disk portion collapse and fit

tightly against the inner walls (compare fig. 162, showing the outer wall uncollapsed in a younger trichome head, and fig. 163, with outer wall collapsed). The singular appearance of such trichome heads, seeming to consist of a layer of open cuplike cells, has been described for other bromeliaceous species (**23, 61, 141**).

The thin walls of the ala cells eventually collapse to form a thin, membranelike, often folded border beyond the periphery of the disk portion just described. It is usually difficult to see this collapsed ala portion of older trichome heads in a surface view of a leaf, since it becomes almost invisible, lying pressed against and sometimes coalescent with the underlying trichome heads or epidermal cells which are visible through it. The disk portion, on the other hand, is quite distinct even when a layer of air is not present beneath it, because of the thick lateral, i.e., radial, especially, and tangential, walls of its cells which give it the appearance of a ribbed, umbrella-like structure.

The solid membranous cones which appear on the underside of the trichome heads and which fit into the anterior chambers of the stomata, reported by LINSBAUER (**88**) as occurring in some bromeliaceous species, were not found in the pineapple. An operculum-like trichome head, characterized by an extraordinary thickening of the outer walls of cells and which is found in all species of *Tillandsia*, according to TIETZE (**150**), is also absent in pineapple; SOLEREDER and MEYER (**141**) reported that the operculum is always absent in the tribe of *Bromelia* to which the pineapple belongs.

Upon superficial examination it appears that the upper surface of the pineapple leaf is free of the scurfy trichome covering so characteristic of the lower surface. This is not only because there are only half as many trichomes on the upper as on the lower surface but for other reasons as well. Because of the absence on the upper side of the deep furrows and ridges found on the lower side, the trichome heads lie more closely appressed to the epidermis, and there is little or no air space beneath them. The air beneath the heads on the lower leaf surface gives this surface its silvery-white appearance. Also, the cells forming the heads on the upper surface are somewhat smaller and have comparatively thinner radial and tangential walls than those of the heads on the lower surface. Eventually many of the trichome heads on the upper surface of older leaves become detached, as a result, perhaps, of weathering (figs. 164, 165). This loss of the heads is, of course, another reason for the glabrous appearance of the upper surface of the leaf.

FUNCTIONS.—The function of absorption of water and aqueous nutrient solutions is the one most often stressed in descriptions of the trichomes of Bromeliaceae. In the epiphytic species of this family, of which *Tillandsia usneoides* is the most striking and noted example, the trichome is of great importance in this function. Many of the early investigators (**22, 61, 95, 110, 118, 120, 131**), however, already realized that these structures serve another purpose—they contribute toward the reduction of transpiration. In the pineapple leaf the latter function is probably the more important of the two.

That the trichomes on both surfaces of the basal portions of the pineapple leaf may absorb water and aqueous nutrient solutions seems not too improbable, since these particular trichomes consist of living cells and, in addition, have access to an almost constant supply of water from rain and dew which collects between the stem and ensheathing leaf bases. Several

previous writers (**68, 107, 118, 126**), although implying or stating that the trichomes on all parts of the leaf are more or less functional in pineapple, described these structures as being more numerous and able to absorb water on the basal portion of the leaf. There is no definite proof available, however, that the trichomes on the base of the leaf do absorb water and dissolved nutrients.

The trichomes on the lower surface of the leaf blade proper appear unwettable. Drops of water placed on this surface do not spread but remain unabsorbed for experimental periods of 3–6 hours and finally disappear, apparently as a result of evaporation rather than absorption. This postulation is substantiated by the observation that the surface of the leaf seen through the drop of water remains unaltered in appearance—it retains its silvery-white color owing to the air present within and beneath the trichome head—whereas on the leaves of such plants in which the trichomes definitely function as absorbing hairs (e.g., *Tillandsia usneoides*) the silvery-white color changes immediately to a pure green as a result of the visibility of the leaf through the layer of absorbed water (**118**). Furthermore, similar drops of water placed on experimental nonabsorbent surfaces disappeared, obviously as a result of evaporation, at approximately the same rate as from the lower surface of the pineapple leaf. Earlier investigators (**110, 167**) had come to a similar conclusion on the nonabsorptive ability of the trichomes on the undersurface of the blade proper of terrestrial Bromeliaceae, including *A. comosus*.

The trichomes on the upper surface of the blade proved experimentally just as unwettable as those on the lower surface of the pineapple leaf. On the other hand, RICHTER (**110**) reported that the upper surface of leaves of certain closely related terrestrial Bromeliaceae was wettable.

Without differentiating between the trichomes on the upper and lower surfaces, or between the base or blade of the leaf, other reports on water absorption are variable. ASO (**6**) stated that detached leaves of *A. comosus*, with cut ends sealed, absorbed water but not solutions of lithium nitrate or potassium ferricyanide. If the dissolved lithium salt was placed in the axils of leaves of intact plants, negligible amounts of lithium were found within the leaves. SCHULZ (**126**) found the trichomes wettable and concluded that this was definite evidence that they absorbed water. SIDERIS (**131**) assumed that the trichomes on the pineapple leaf absorb water because similar structures are known to absorb water in other Bromeliaceae. He and other workers in the pineapple industry in Hawaii have at various times reported the absorption of iron sulfate solution when placed on the blade of chlorotic pineapple leaves, as evidenced by the greening of the localized spots to which the solution was applied. This type of application has not, however, been subjected to careful experimentation.

PITTENDRIGH (**107**) believed that water absorption by *A. comosus* is dependent upon three supplementing systems: the soil roots, the "interfoliar" (axillary) root system, and the "epidermal trichomes." Other authors, including RICHTER (**110**), merely assumed that in terrestrial Bromeliaceae, including *A. comosus*, which have an abundant soil root system, water absorption by the trichomes is unnecessary. BERGMAN expressed the opinion, in a private communication, that the function of water absorption has been lost by the trichomes in this species in the course of phylogenetic progress within the family, al-

though the modern conception is that the terrestrial Bromeliaceae are the more primitive form (107).

The detailed study by MEZ (95) remains the classic work on the mechanism of water absorption by the trichomes in the Bromeliaceae and has been referred to by all authors who have written on this subject. Essentially, the trichome functions as follows: the dry (air-filled) trichome head serves as a capillary organ and, when wetted, expands, setting up first a suction-pump mechanism drawing water into a dead (head) cell apposed to the stalk of living cells through which the water is then drawn osmotically to the mesophyll. The absorbing hair is thus an effective one-way valve (107).

As a supplementary protection against transpiration the trichomes play a far more important role in the water economy of the pineapple plant than in water absorption, if they do serve the latter function. The overlapping trichome heads form a more or less dense covering over the stomata and consequently reduce the loss of water vapor from these structures considerably. Moreover, the quiet layer of air which lies between the lower surfaces of the trichome heads and the epidermis must become saturated with water vapor from the stomata, and this serves as a further deterrent to transpiration. Others (95, 110, 118, 120, 142) have drawn attention to this function of the trichome head in other bromeliaceous species.

It has been proposed that the trichome heads serve as a protection against intense sunlight. BAUMERT (12) demonstrated, with the use of thermoneedles and a galvanometer for reading, that the dispersion of light incident to bromeliaceous leaves covered with dry trichomes was 23.8% greater than from these leaves where the trichomes were removed. SIDERIS (131) also suggested the importance of the trichomes in light reflection. STAUDERMANN (142) referred to BAUMERT's work; he believed that the thick radial walls of the trichome head are involved in the light dispersion. STAUDERMANN thought that the dry trichome heads also serve as a protection against sudden great changes in temperature in the leaf, since the air-filled cells are poor conductors of heat.

That the trichomes are of a "glandular nature"—in the sense of an excreting organ—as MILES THOMAS and HOLMES (98) suggested in their description of the seedling pineapple plant, is not true.

FILIFORM HAIRS

The various modifications of the peltate scutiform trichomes described in the preceding section represent the only hair form occurring on the leaf of a mature pineapple plant. On the leaves of seedlings, however, there occur unbranched multicellular filiform hairs. Since MILES THOMAS and HOLMES (98), who made an extensive study of the seedling and young plant of the pineapple, made no mention of this hair, and no other reference to it has been found in the literature on the Bromeliaceae, a brief description will be presented.

These hairs occur on both surfaces and, in greatest numbers, on the margins of the leaf. They are not, as a rule, visible to the naked eye, as are the scutiform trichomes, but, if a seedling leaf is held up against the light, the marginal hairs give it a finely toothed appearance. Although the hairs appear more or less equally spaced along the margin, they are irregularly and rather sparsely distributed over the two surfaces. They appear on the first leaf of the seedling and are most abundant on about the third and fourth leaves. On successively formed leaves the

scutiform trichomes begin to appear in small numbers. The number of the latter is increasingly greater on later-formed leaves and the number of the filiform hairs fewer, so that on about the tenth leaf none of the latter is to be found.

A study of the development of these hairs shows the following stages. An epidermal cell which is the direct initial or mother cell of the hair begins to expand slightly in somewhat the same manner as the trichome mother cell already described—the distended portion appears as a hemispherical structure protruding beyond the surface of the epidermis (fig. 179*A*, *B*). In the next stage a transverse wall forms in this mother cell at the level of the outer wall of the adjacent epidermal cells (fig. 179*C*). The acroscopical cell of the two daughter cells thus produced elongates somewhat in a direction at right angles to the leaf surface before the first of a series of transverse walls form (fig. 179*D*). Elongation of these cells and further division by a series of transverse walls progress rapidly until a filament consisting of from four to five tubular-shaped cells is formed. This is the body[26] of the hair. Although the cells which form the body are more or less similar in shape and size during the earlier stages of development of the filament (fig. 179*D*), they differ somewhat from one another in the mature hair.

Before, or more often soon after, the first transverse wall forms in the daughter cell from which the body of the hair originates, an anticlinal wall forms in the basiscopic daughter cell (fig. 179*J*); occasionally, two anticlinal walls develop (fig. 179*I*). The two—or three, in the latter case—cells thus formed adjacent to each other, and in about the same transverse plane, constitute the foot of the hair.

During the period in which the body and foot are developing, the epidermal cells adjoining the foot and in a line continuous with it grow more rapidly than the foot itself, so that a depression is formed into which the basiscopic cell of the body fits (fig. 179*K*, *N*).

When fully developed, the typical filiform hair has the following appearance. As seen to best advantage in a vertical median view, the foot of the hair, consisting of two or three small cells, lies at the base of a depression in the epidermis (fig. 179*K*). Into this depression, lined by epidermal cells which are narrower and longer in this view—their long axis is greater and their short axis less—than the mass of epidermal cells, the basiscopic cell of the body fits closely. The next cell, acroscopic to the basal cell just described, has a greater diameter than the other hair cells and, because it is practically isodiametric, appears bulblike (fig. 179*N*). In some cases the next cell acroscopic to it is also bulblike with either a greater diameter than the cell just described (fig. 179*N*) or a lesser diameter. If not bulblike, this last-described cell, and the next one acroscopic to it, are tubular or semitubular, i.e., a transitional type between a bulblike and tube shape. The apical cell in the first-formed leaves is tubular in shape with a rounded free end (fig. 179*O3*), but in later-formed leaves the free end of this cell is distended bulblike, often with a slight constriction in the diameter basiscopic to the swollen end (fig. 179*O1*, *2*).

During development the hair cells are filled with a clear protoplasm which persists for a considerable time but eventually dries up, apparently, and lies close against the walls. The apical cell often

[26] The terminology used here and in the following paragraphs is that of DeBary (30) for hairs of a similar type in other plants; the terms have been generally adopted in descriptions of hairs.

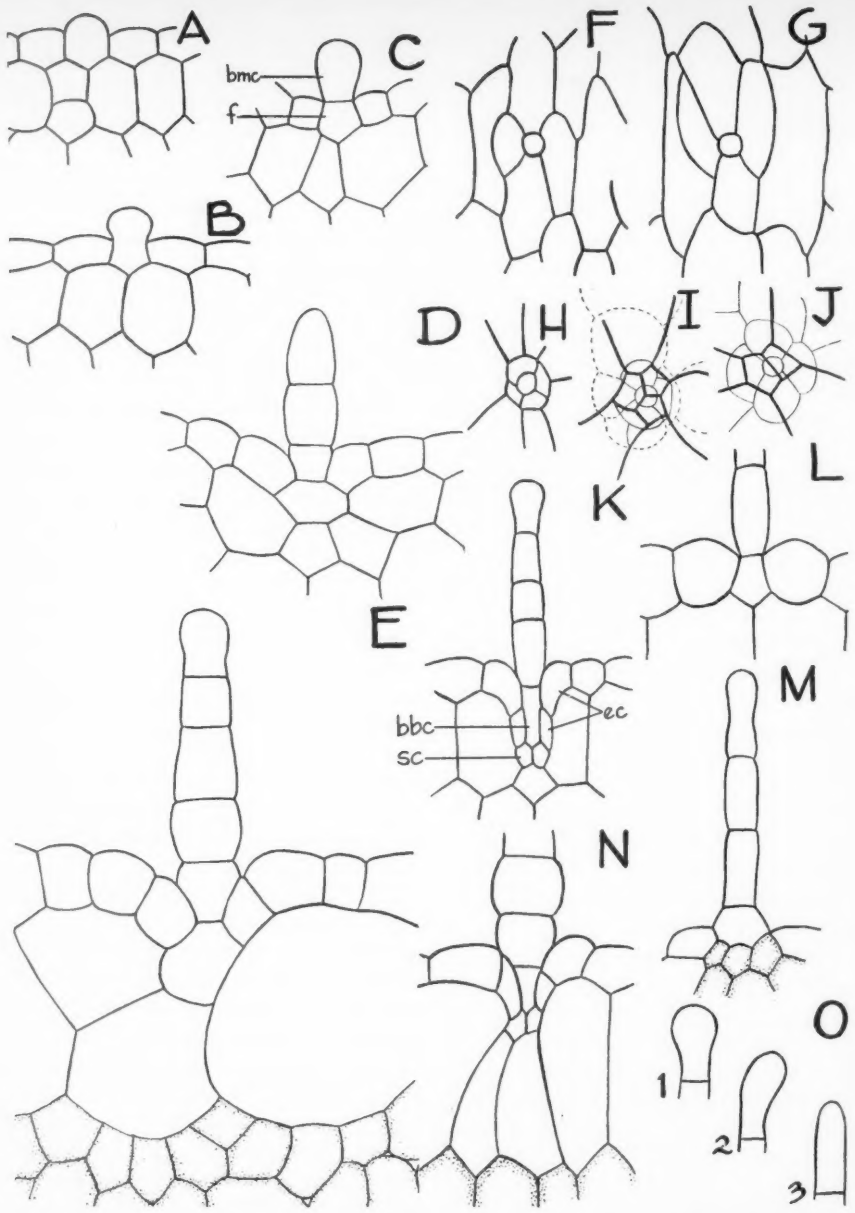


FIG. 179.—Filiform hair. *A–E*, vertical views of successive stages in development of simple filiform hairs, found only on first-formed seedling leaves. *A*, epidermal cell enlarges to form expanded portion above surface of epidermis (*B*). Then periclinal wall forms at level of outer wall of adjacent epidermal cells to cut off basiscopic cell (*C*) which becomes foot of hair (*f*), and acroscopic cell which becomes mother cell of hair body (*bmc*). Successively formed periclinal walls in body mother cell results in single vertical row of cells which make up complete hair body (*D*, *E*). *F–J*, paradermal views of hair and subsidiary cells of type shown in vertical view in *K* (hair type most usual on upper and lower surfaces of leaf): at level of epidermis (*F*) and at successively deeper levels—*G*, slightly below epidermal level; *H*, at level of basiscopic body cell (*bbc* in *K*), with surrounding subsidiary (epidermal) cells (*sc* in *K*); *I*, *J*, at level of foot and modified hypodermal cells (observed from below foot); cells at level above foot shown with lighter or broken lines. *L*, *N*, variations of hair bases. *M*, type of hair found at margin of leaf. *O*, *1–3*, types of apical cells of hair body: *3*, in first-formed; *1–2*, in later-formed leaves. Stippled cells are chlorophyllous cells. *A–E*, ×350; *F–O*, ×175.

collapses or is torn off entirely on the oldest leaves.

The significance of these hairs is unknown, and the scope of this work did not justify any further investigation. They should probably be grouped with those structures described by GOEBEL (**49**) as belonging to the "juvenile stage" of certain plants.

MARGINAL SPINES

The Smooth Cayenne variety of pineapple derives its name from the smooth margins of its leaves. The variety is characterized, however, by a spiny tip on all of its leaves and occasional spines at various points along the margins of some of them (fig. 6*B*, *C*). COLLINS (**26**) has recently pointed out that the action of the gene for development of the spiny tip appears to be associated with the physiological conditions which initiate leaf growth. After the growth processes are under way, the action of this gene for spine production is inhibited. At any period after initiation some environmental factor (nutrition, drought, etc.) may cause a cessation in the growth of the leaf. Upon the resumption of growth the activity of the gene responsible for spine formation is renewed, and spines are formed for a short period of time before the inhibiting action takes place again. This accounts for the scattered groups of one or more spines at various intervals along the margins of fully developed leaves (fig. 180*G*, *H*).

Examination of very young leaves shows that the spines at the tip originate in the marginal region of the apical meristem as marginal projections early in the development of the leaf (fig. 173). Although most of the spines originate as single projections, occasionally they arise doubled or tripled—as a pair or three having a common base (fig. 180*A*–*F*). The tissue comprising the projection remains meristematic for a longer period of time than the adjacent marginal and blade regions. In the further development of the spines the meristematic tissue is differentiated into an epidermis, a hypodermal layer, and, inclosed by these layers, a mass of undifferentiated mesophyll fairly rich in chloroplasts.

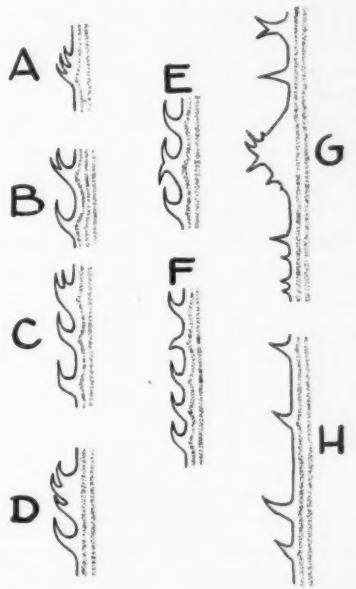


FIG. 180.—Marginal spines. *A*–*F*, variations in types at tip, and *G*–*H*, at base of leaves. Direction of leaf apex toward top of page. Stippling indicates furrow on leaf under surface. About ×2.5 natural size.

In the mature state the spines project approximately 1 mm. beyond the margin of the blade. The spine has a base in the form of an equilateral triangle, with a sharp, usually narrowly drawn-out, rigid point which is hooked, the tip directed toward the apex of the leaf (fig. 180*A*–*C*, *F*). This last characteristic is not without exception, however, since, rarely, one finds spines whose tips point toward the base of the leaf (fig. 180*E*).

Since the differentiation of the various tissues within the meristematic projection is similar to that in the leaf primordium already presented, a description of the development of the spine is omitted.

The epidermal cells of the mature spine, consisting of a single layer continuous with that of the margin and blade surfaces, resemble the cells of the latter, although greatly elongated in the direction of the spine tip. The lateral and inner walls of these cells are also sinuous and greatly thickened, and the cells contain silica bodies. Scattered stomata and scutiform trichomes occur.

The hypodermal layer at the base of the spine consists of several rows of sclerenchyma cells, as at the margin of the leaf, while the tip of the spine is formed of a solid mass of hypodermal cells beneath the inclosing epidermis. The color of the suberized walls of these hypodermal cells gives the reddish-brown appearance to the tips of mature spines. The hypodermal cells at the tip are considerably shorter than those in the basal portion of the spine and in the rest of the leaf.

The mass of the basal portion of the spine, i.e., inclosed within the epidermal and hypodermal layers, is occupied by a mesophyll similar to that found in the assimilatory tissue of the leaf proper. The chloroplasts of these cells are visible through the hypodermal and epidermal layers, so that the basal portion of the spine appears green in contrast to the brown tip.

DEBROUCK (31) and UHLWORM (153) have briefly described spines in bromeliaceous plants.

[*To be concluded*]

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON ROOT DEVELOPMENT IN YOUNG CORN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 604

MARY AILEEN MURRAY AND A. GERALDINE WHITING

Introduction

As a selective herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D) has shown remarkable promise for weed control in the corn crop (16). Proper application for effective control, however, is complicated by the fact that the corn plant itself may be sensitive to 2,4-D. Both published (1, 3, 9, 10, 13, 17, 20) and verbal reports indicate that injurious effects may occur in various stages of growth from emergence of seedling to development and maturation of tassel and ear. Many factors appear to influence the susceptibility of the corn plant (9). Among these are rate and time of application, soil factors, climatic conditions, and age and variety of the corn being treated. As a consequence of these many variables, the results both of field use and of preliminary controlled experiments are often inconclusive and are not always in agreement.

The present paper reports histological investigations of the effects of 2,4-D on root development in young sweet-corn plants (*Zea mays* L.). BUCHHOLTZ (9) reported that excessive root proliferation was one of the common 2,4-D injuries in corn. HAMNER *et al.* (10) observed the formation of increased numbers of roots

following application of the sodium salt of 2,4-dichlorophenoxyacetic acid to the soil as a pre-emergence spray but inhibition of the brace roots when the herbicide was used as a foliar spray. ELLIS, in a reference by LEE (13), noted both stunting of brace roots and increased root growth resulting from foliar sprays used on sweet corn. BUCHHOLTZ (9) has reported that greatest root injury occurred when corn plants between 10 and 40 inches in height were treated, but others (4, 13) have tentatively recommended spraying the plants at heights varying from 12 to 36 inches. Since the character of the root system in the corn plant is commonly considered to be a major factor in determining the mature size and yield of the plant (14), any modification of this system is of significance in establishing the usefulness of this new herbicide to the corn-grower.

Material and methods

Seven varieties of sweet corn (Bantam Evergreen, Early Evergreen, Golden Bantam, Golden Cross, Ioana, Marcross, and Spancross) were selected for experimentation. Varietal differences in sensitivity (9, 13) were expected, with possible accompanying differences in the character of response. In order that at least preliminary observations and histological collections could be made of field-grown material, seeds were row-planted in the garden on September 8 when work on the experiment was begun. Eighteen days after planting the young corn was about 9 inches high, with the first four or five foliage leaves expanded and others rolled in the bud. These young plants were sprayed with a 1% water-oil emulsion of the butyl ester of 2,4-D applied with a DeVilbiss atomizer. The spray was made from the commercial spray "Weed-No-More" supplied by the Sherwin-Williams Company. By computation, about 0.1 ml. of spray was used per plant. Although spray was directed at all parts of the plant, the foliage intercepted the greater amount and was sprayed to wetness without, if possible, the accumulation of heavy or running drops. Observations and histological collections were made for a period of 5 weeks after spraying. During this time the weather gradually became unfavorable. Work was then transferred to the greenhouse.

A second lot of plants was grown in the greenhouse in the fall and a third lot in the spring. The fall series included five varieties (Early Evergreen, Golden Bantam, Golden Cross, Ioana, and Marcross) and the spring series the single variety, Early Evergreen. In both series methods similar to those initiated in the garden were followed. Plants were grown in 8-inch pots, with seven or eight seedlings per pot at the time of spraying and with gradual thinning to two per pot. These plants were sprayed when they reached the same stage of development as the garden plants at the time of spraying (with the fourth or fifth foliage leaf expanded). Approximately the same amount of spray per plant was used. Observations and histological collections were made over a period of five weeks. The spray applied to plants with four or five leaves expanded will be referred to as the "early spray on young plants."

Some of the plants of Early Evergreen and Golden Bantam grown in the greenhouse in the fall series were not treated at the same time that the other plants were sprayed but received the same spray 3 weeks later. In these older plants the seventh or eighth leaf was expanded and the rolled tip of the next leaf was showing in the bud. The variety Early Evergreen was about 12 inches tall and Golden Ban-

tam about 16 inches tall. It was determined by dissection that the tassel was formed at the apex of the stem, whereas at the time of the early spray the apical meristem of the young plants showed only leaf primordia. General observations and histological collections were also made of this older series. The spray applied to plants with seven or eight leaves expanded will be referred to as the "later spray on older plants."

Observations were recorded for all varieties treated both in the garden and in the greenhouse. Histological examination was limited to two varieties—Golden Bantam, which was large and vigorous in growth, and Early Evergreen, which was smaller and more slender. Both were representative, however, of the general character of response found in the seven varieties grown. Early Evergreen has been selected for detailed description in this report. Histological specimens were preserved in Navashin's solution, imbedded by the butyl alcohol–paraffin method, and sectioned at 12 or 15 μ. A modified Flemming's triple stain was used.

Results

General observations

The foliage was the first part of the young corn plant to show the effects of the spray. At the end of 24 hours the bright green color of the leaf, especially along the margins and at the tips of the blades, had dulled to a translucent, olive-green color. Within a day or two more, the injured areas were brown and dying. These changes occurred in the expanded leaves of the treated plants both in the garden and in the greenhouse. The young, rolled leaf blades showed no response except in the older corn treated with the later spray in the greenhouse. In these plants the outer young leaf, rolled in the bud at the time of spraying, often failed to unfold, and its margins became puckered. Still younger leaves were retained within, and their growth was checked by this affected leaf.

The stem orientation of the plants in the garden, with their growth slowed by low temperatures, was not affected by the spray. Some plants in the early treatment in the greenhouse exhibited a tendency to bend at some of the lower nodes. The older plants treated in the seven- to eight-leaf stage showed a similar tendency, but the bending occurred two or three nodes higher on the stem. In general, the whole top growth of the plant was stunted by the treatment with 2,4-D in comparison with growth in the untreated plants.

The most significant effect of treatment in the young corn plants with four or five leaves expanded was the response of the lower internodes of the stem and of the adventitious brace roots which develop at the bases of these internodes to form the permanent root system of the corn plant. At the time of spraying the first whorl of brace roots (above the second node from which the coleoptile diverges) was already developed (table 1). The second whorl (third node—first foliage leaf) was partially developed and continued to grow normally. The extent of development varied from plant to plant. Likewise, the third whorl of roots (fourth node—second foliage leaf) showed variation in its growth, but in many plants its formation had begun before the time of spraying. By 2 weeks after spraying the roots of this whorl were well extended. In each higher whorl the roots were larger than in the previous whorl and were generally three or four in number. This development was similar to that occurring in the controls. In some plants, however, root development in the

third whorl showed malformation (fig. 1*C*) resulting from treatment. Root tips emerged not as single roots but as fasciated groups of roots. These structures might become unusually massive and were often without the downward orientation of roots. Soil particles strongly adhered to the surface of these fasciated roots which were gelatinous to the touch.

The greatest effect of treatment was generally found in the fourth whorl of roots (fifth node—third foliage leaf). Swelling at this position was perceptible at the end of the first week after spraying. Enlargement occurred around the entire circumference of the stem (fig. 1*A*, *C*, *D*) rather than at the three or four points usual to root formation. Growth

TABLE 1

SUMMARY OF RESPONSE IN CORN PLANTS RECEIVING FOLIAR SPRAYS OF 1% OF BUTYL ESTER OF 2,4-D AT TWO DIFFERENT STAGES OF GROWTH

Nodes of stem	Leaves	Whorls of adventitious roots	Early spray on young plants (four leaves expanded)			Later spray on older plants (seven leaves expanded)		
			Leaf development at time of spraying	Root development at time of spraying	Response* after spraying	Leaf development at time of spraying	Root development at time of spraying	Response* after spraying
10	Eighth					Rolled in bud		Leaf remaining rolled in bud
9	Seventh					Expanding		
8	Sixth					Expanded		Sometimes nodal enlargement, incipient root formation, fasciation
7	Fifth		Rolled in bud			Expanded		Marked nodal enlargement; some incipient root formation, fasciation
6	Fourth	5	Expanding	Undeveloped	Sometimes roots fasciated	Expanded	Sometimes partially initiated	Sometimes root fasciation, or nodal enlargement
5	Third	4	Expanded	Undeveloped	Greatest root fasciation	Expanded	Partly extended	
4	Second	3	Expanded	Initiated or about to be initiated	Frequently roots fasciated	Mature	Extended	
3	First foliage	2	Expanded	Partially formed		Withering	Mature, extended	
2	Coleoptile	1	Withering	Mature, extended		Withered	Mature, extended	
1	Cotyledon							

* Sprayed leaves generally showed some injury. Bending of stem occurred in some plants at nodes showing response in the respective treatments.

gradually increased. By 18 days after spraying in the garden, and by 25 days after the early spray in the greenhouse, many plants showed extreme root fasciation (figs. 1*E*, 2*A*, *B*, *C*). The malformation was similar to but generally exceeded that occurring in the third whorl. In some plants there was a massive collar of upturned tissue (fig. 2*C*). In other plants there were variously divided ribbons of fasciated tissue with or without distal separation into root tips (fig. 2*A*, *B*). Ultimate development of these fasciated root masses differed considerably, perhaps dependent on the degree of fasciation or the relationship to soil line and moisture. In some plants the tissues remained massive with no further division and little indication of functional roots. In other plants numerous—twenty or more—finely divided rootlets arose from the margins or even the surface of these ribbons of fasciated tissue (fig. 2*E*). In still other plants a small number—sometimes as many as ten or eleven—of large and strong roots might develop (fig. 2*D*). In control plants three or four roots ordinarily formed this fourth whorl.

Formation of a fifth whorl of roots (sixth node—fourth foliage leaf) varied considerably. In some plants, both treated and untreated, this tier of roots was not apparent (fig. 12) before the end of the period of observation. In other plants short roots or newly emerged tips were present. Fasciation was common in this whorl among treated plants (fig. 1*E*). The malformation was usually less than that found in the fourth whorl of roots and often consisted of a strongly developed ridge of tissue wholly, or in part, encircling the internode. Although mature field-grown plants average five to seven whorls of brace roots (14), the fifth whorl was the extent of root formation observed during this experiment. It is significant that in these young plants the three whorls of roots showing response, the third to fifth, were associated at the nodes with the second to fourth leaves which were expanded and received the spray.

Treatment of the older plants, with as many as seven or eight foliage leaves expanded, resulted in a less marked response than in the young plants. The location of the response above the sixth, seventh, or eighth nodes (the fourth, fifth, and sixth foliage leaves, table 1) readily distinguished this treatment from the early spray in which response occurred above the fourth to sixth nodes. Two weeks after the later spraying enlargement in these higher internodes was evident (fig. 1*B*). In 5 weeks the ridge-like swellings and the occasional emergence of a root tip above the sixth to eighth nodes suggested disturbances in root formation similar to but less extensive than those at lower nodes in young plants. Infrequently a fifth whorl of fasciated roots at the sixth node was developed in these older treated plants, but the four lower whorls, which were already formed at the time of spraying, grew normally. The greater elongation of these higher internodes in the older plants (fig. 1*B*) placed the responding areas above moisture at the soil surface in an environment proportionately less favorable to root growth.

Histological responses

In contrast to the apparently retarded external response, histological examination indicated that initial changes in cellular activity occurred as early as 2 days after spraying. By the third day certain cells showed marked intensity of cytoplasm and initiation of divisions (fig. 6*B*). These changes correspond with the initiation of proliferation in dicotyledon-

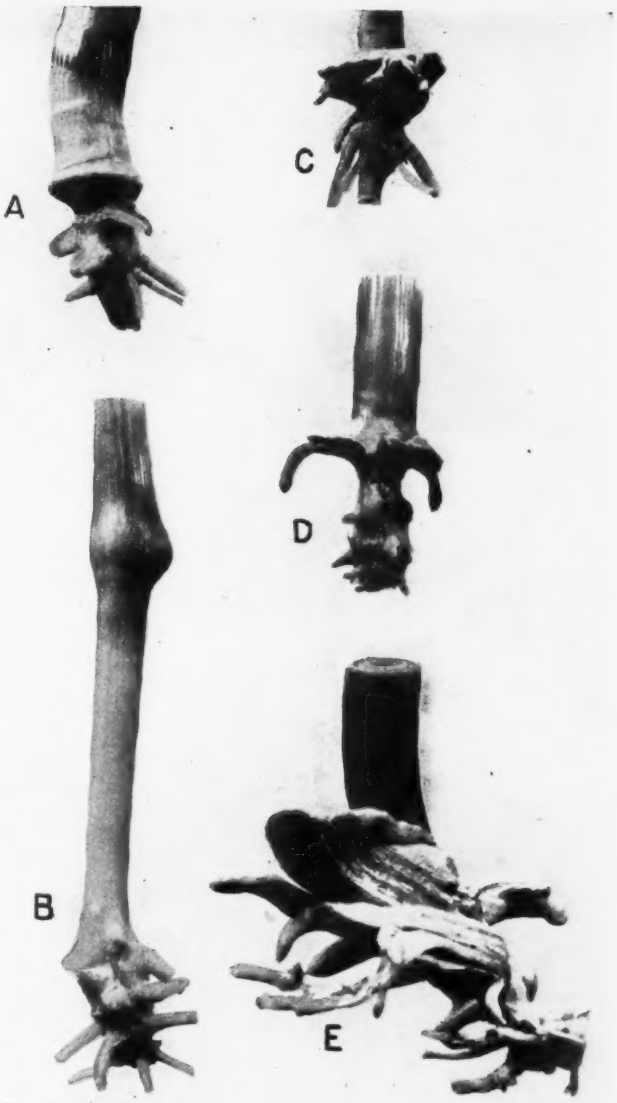


FIG. 1.—Lower portions of stems of corn plants, Early Evergreen, showing lowest whorl of adventitious brace roots above second node and successive whorls of roots above higher nodes. (Normally extended roots cut about 0.5 cm. from stem.) Plants sprayed with 1% concentration of butyl ester of 2,4-D. *A*, *C*, *D*, and *E*, early spray, 16–19 days after planting, on young plants with four or five leaves expanded; *B*, later spray, 37 days after planting, on older plant with seven or eight leaves expanded. (Grown in greenhouse, *A*, *B*, *D*; in garden, *C*, *E*. Collected 21 days after spraying, *B*; 25 days, *C*, *E*; 35 days, *A*, *D*.) Young plants showing response by fasciation in the third (*C*), fourth (*A*, *C*, *D*, *E*), and fifth (*E*) whorls of roots. Older plant showing enlargement at seventh node (*B*); lower four whorls of roots unaffected, possibly slight fasciation in young fifth whorl.

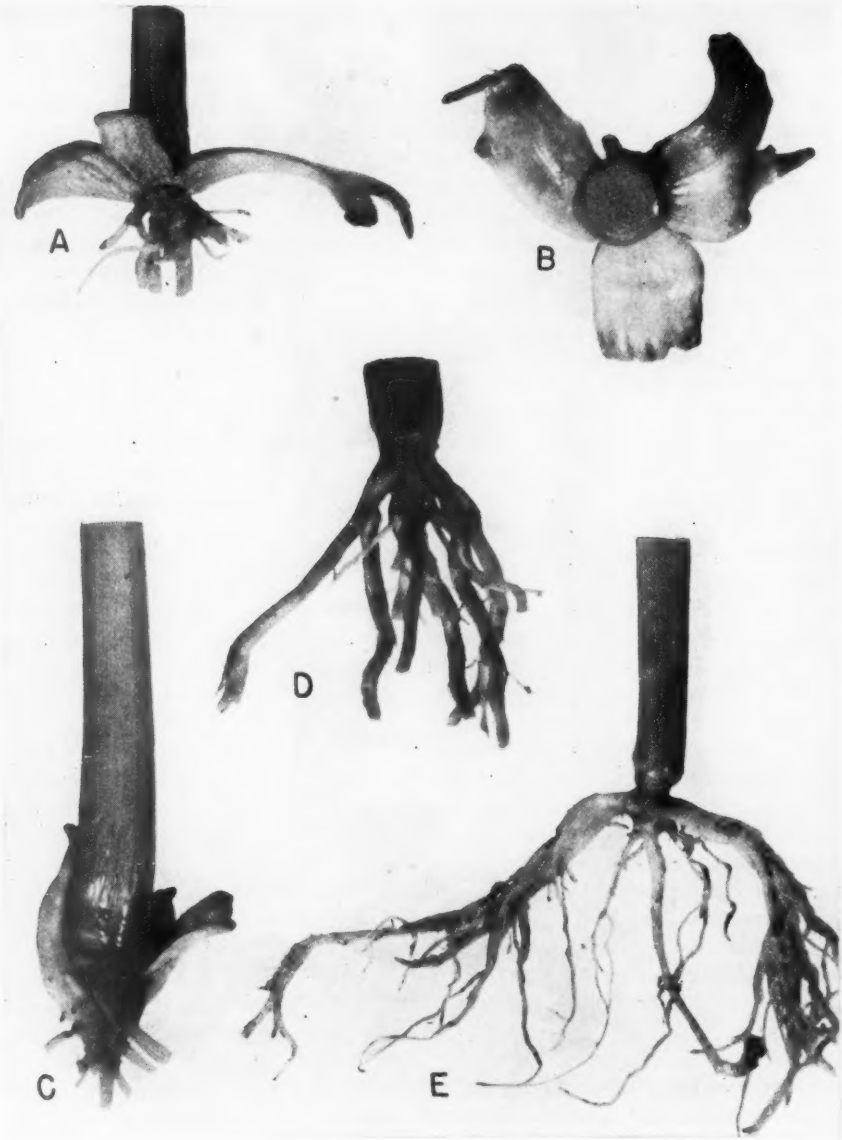


FIG. 2.—Lower portions of stems similar to those in fig. 1. Young plants with four or five leaves expanded when sprayed; grown in garden; collected 25 or 33 days after spraying. *A*, *B*, *D*, Golden Bantam; *C*, Spancross; *E*, Golden Cross. Characteristic variations in fasciation: massive collar of upturned tissue (*C*); broad ribbons of fasciated tissue with some distal separation into roots (*A*, *B*); less fasciation with development of numerous small rootlets (*E*); slight fasciation and formation of number of large functional roots (*D*).

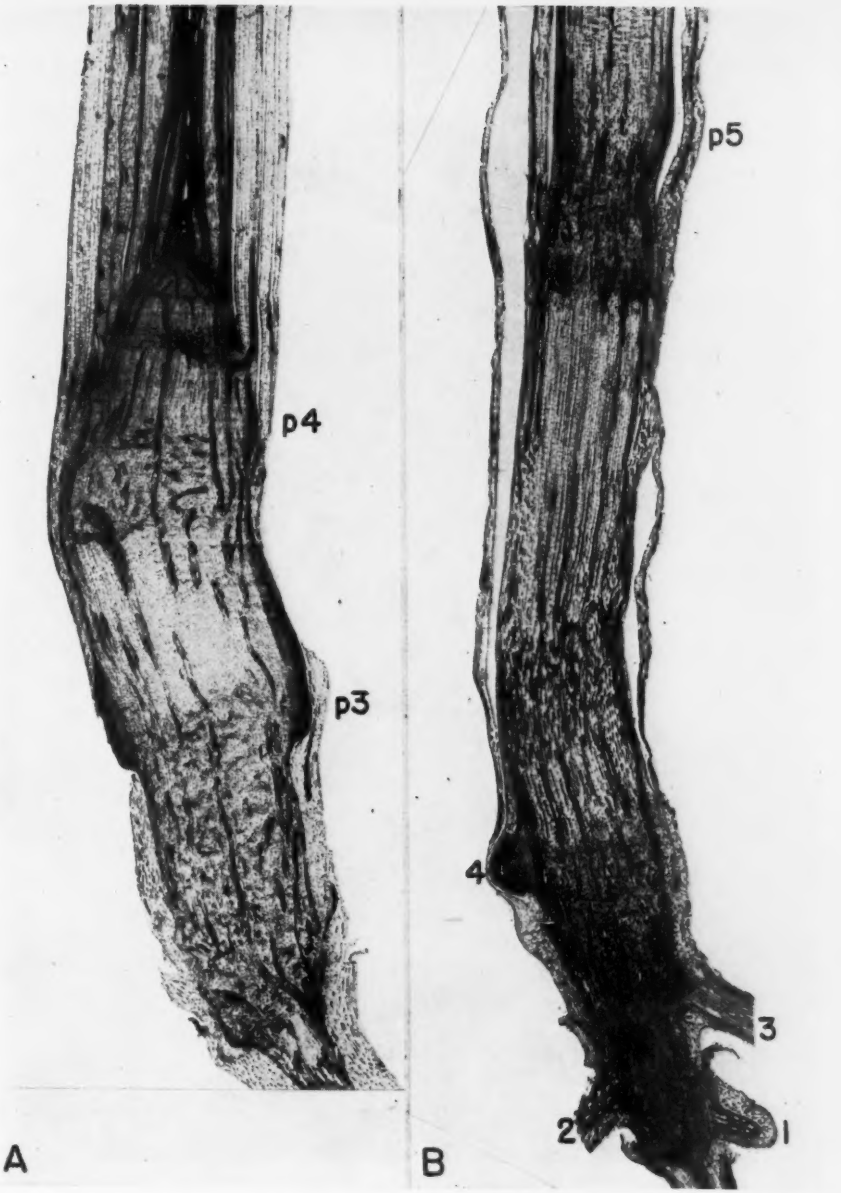


FIG. 3.—Longisections of plants of Early Evergreen (as are all subsequent figures). *A*, young plant showing proliferation of stem tissues at positions of third (p_3) and fourth (p_4) whorls of roots above fourth and fifth nodes; first and second whorls of roots (not shown in section) developed normally. *B*, older plant showing proliferation above sixth node (p_5), but normal root development of first to fourth (1–4) whorls at second to fifth nodes. Note root primordium in fourth whorl above fifth node. Compare elongation of internodes in young plant with older plant. (*A*, 7 days after early spray; *B*, 14 days after later spray; both grown in greenhouse.)

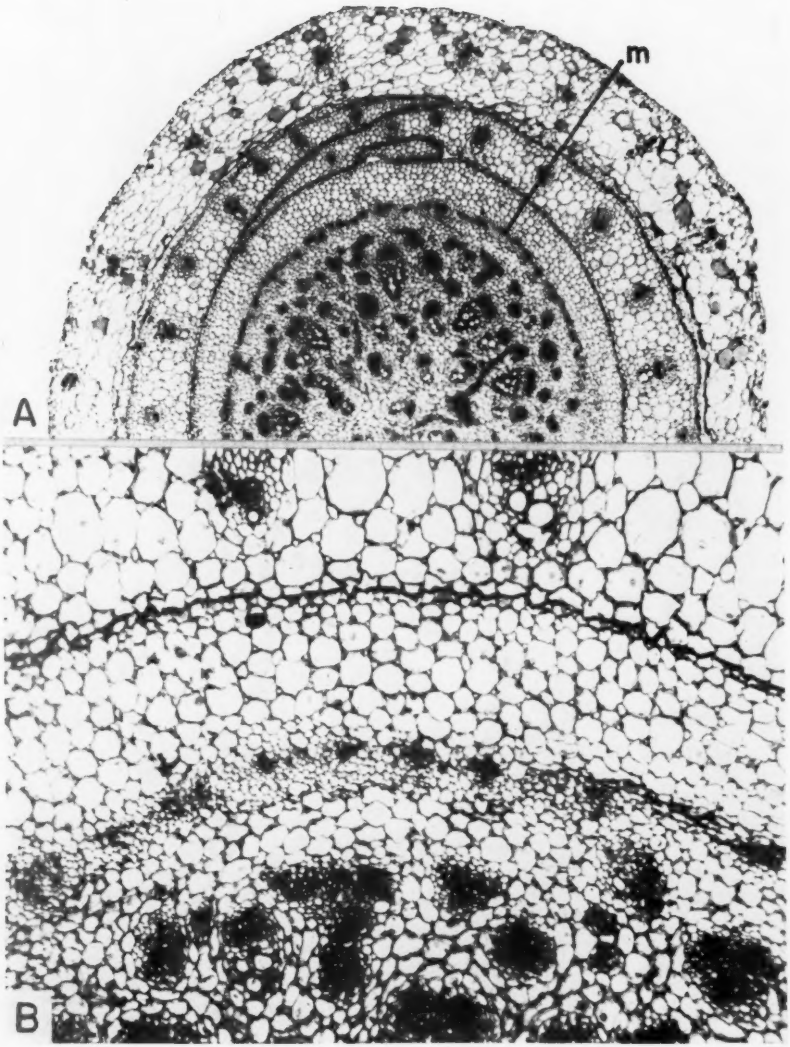


FIG. 4.—Transections of fourth internode of untreated stem, 16 days after planting (at time of early spray). *A*, note position of meristematic zone (*m*) at periphery of stelar tissues and centrad to cortical parenchyma. *B*, detail of *A;* meristematic zone composed of small cells, including some differentiated vascular elements in darker patches.

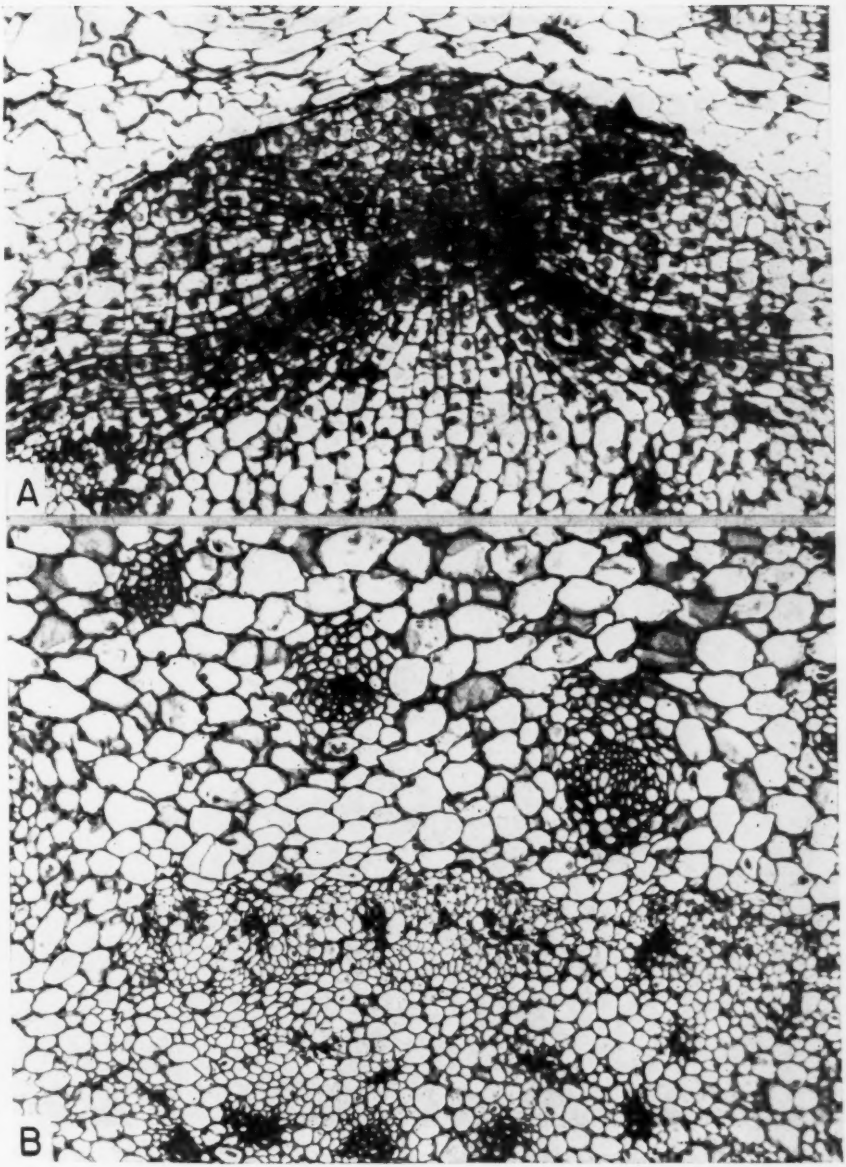


FIG. 5.—*A*, transection of primordium of adventitious root, showing precise organization of histogens: in center the single-layered dermatogen-periblem giving rise to epidermis and cortex; above, calyptrogen originating root cap; below, plerome developing central cylinder of stelar tissues. *B*, transection in fourth internode of young plant, 3 days after spraying. Meristematic zone unaffected by treatment. Compare with fig. 6*B* in which proliferation has commenced in same tissues on opposite side of same stem.

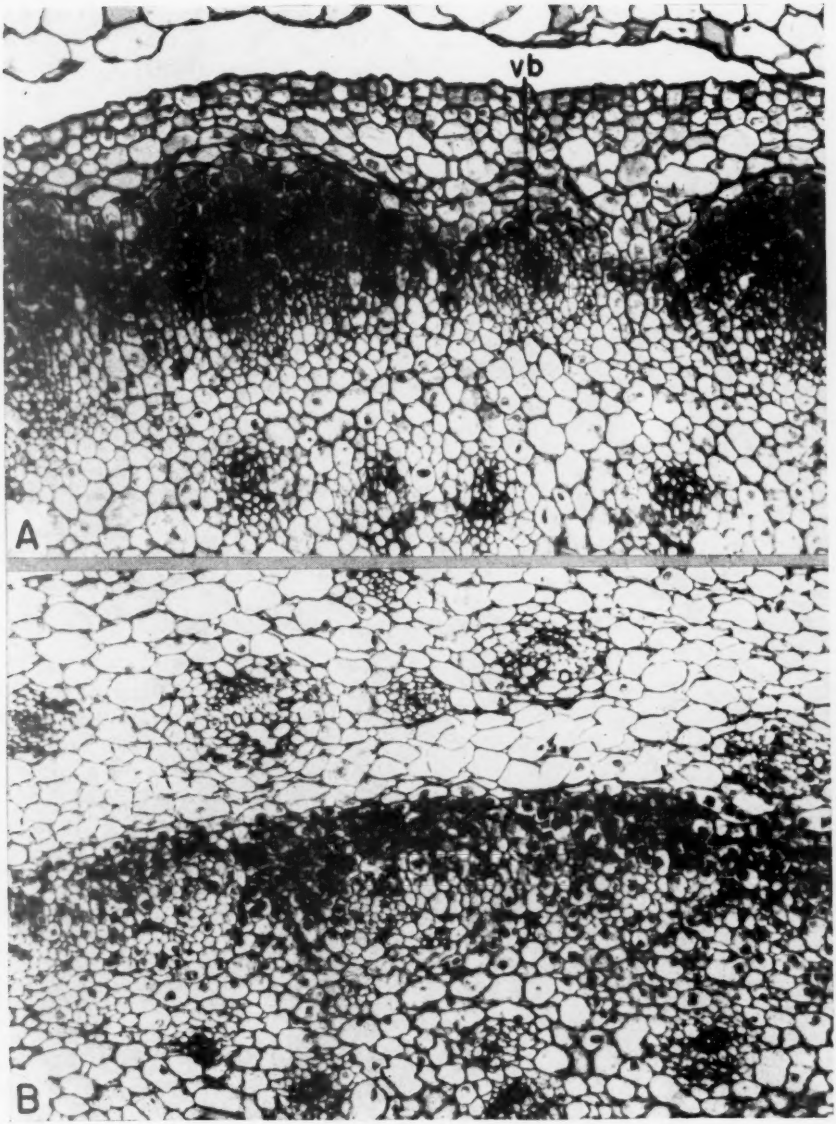


FIG. 6.—*A*, initiation of proliferation in cells lying immediately outside young vascular bundle (*vb*) and in adjacent rays: conspicuous nuclei, dense cytoplasm, and initial tangential cell divisions. On either side of *vb* more advanced stages with cells highly meristematic and dividing rapidly. *B*, parenchymatous cells of meristematic zone proliferating to form band; patches of provascular tissue still inactive. Note sharply defined outer limit of proliferation. (*A*, transection in sixth internode of older plant, 7 days after later spray. *B*, transection in fourth internode of young plant, 3 days after early spray; same stem as *5B*.)

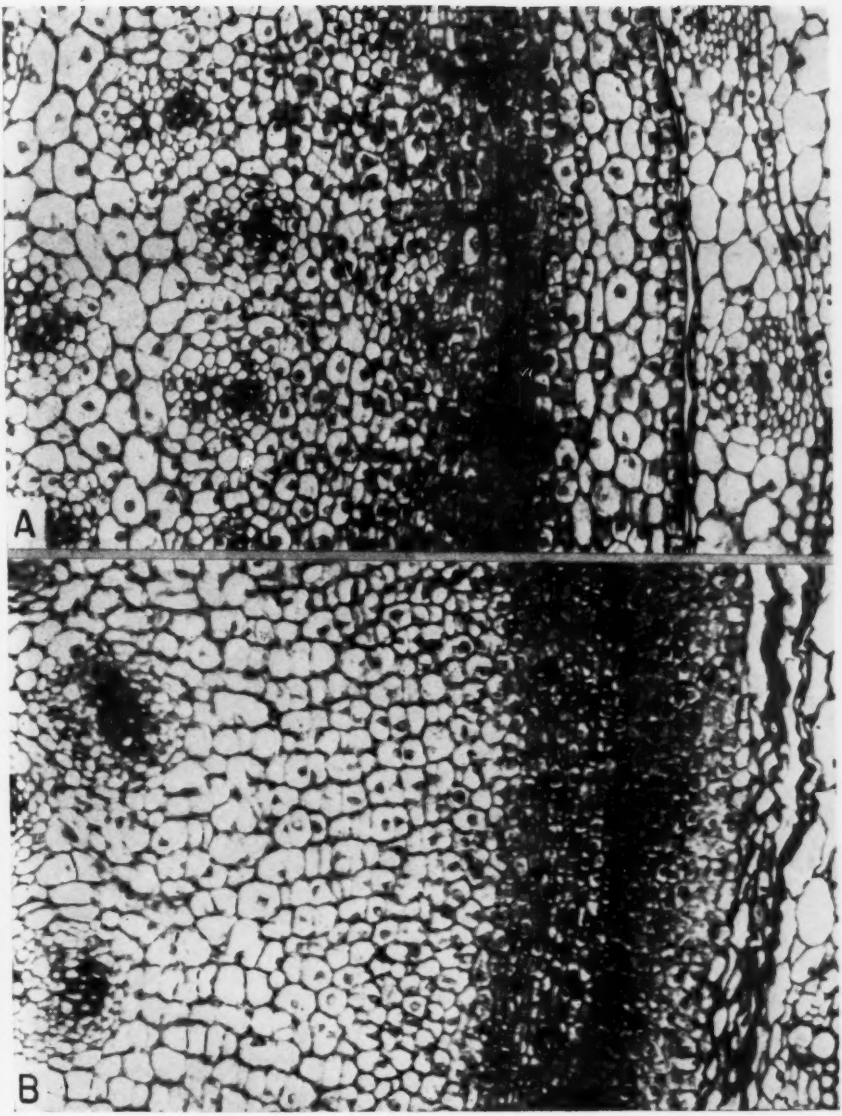


FIG. 7.—*A*, early stage of proliferation; activity general in meristematic zone and increasing in parenchymatous cells centrad to meristematic zone. *B*, later stage showing organization of three regions in area of proliferation: outer region, centrad to crushed cortical parenchyma, cells meristematic; narrow middle region, cells highly meristematic, divisions in tangential plane; inner region, extending to vascular bundles, cells larger and less dense, tangential divisions resulting in radial rows of cells. (Transections in fifth internodes of young stems; *A*, 3 days, *B*, 7 days after early spray.)

FIG. 8.—*A*, proliferated tissues forming continuous band around periphery of stem. Vascular bundles and fundamental parenchyma showing no evident response. *B*, later stage in proliferation; three regions wider, especially inner one, but retaining characteristic pattern. (Transections of young stems, 7 days after early spray; *A*, fifth internode; *B*, fourth internode. *A*, same stem as fig. 7*B*.)

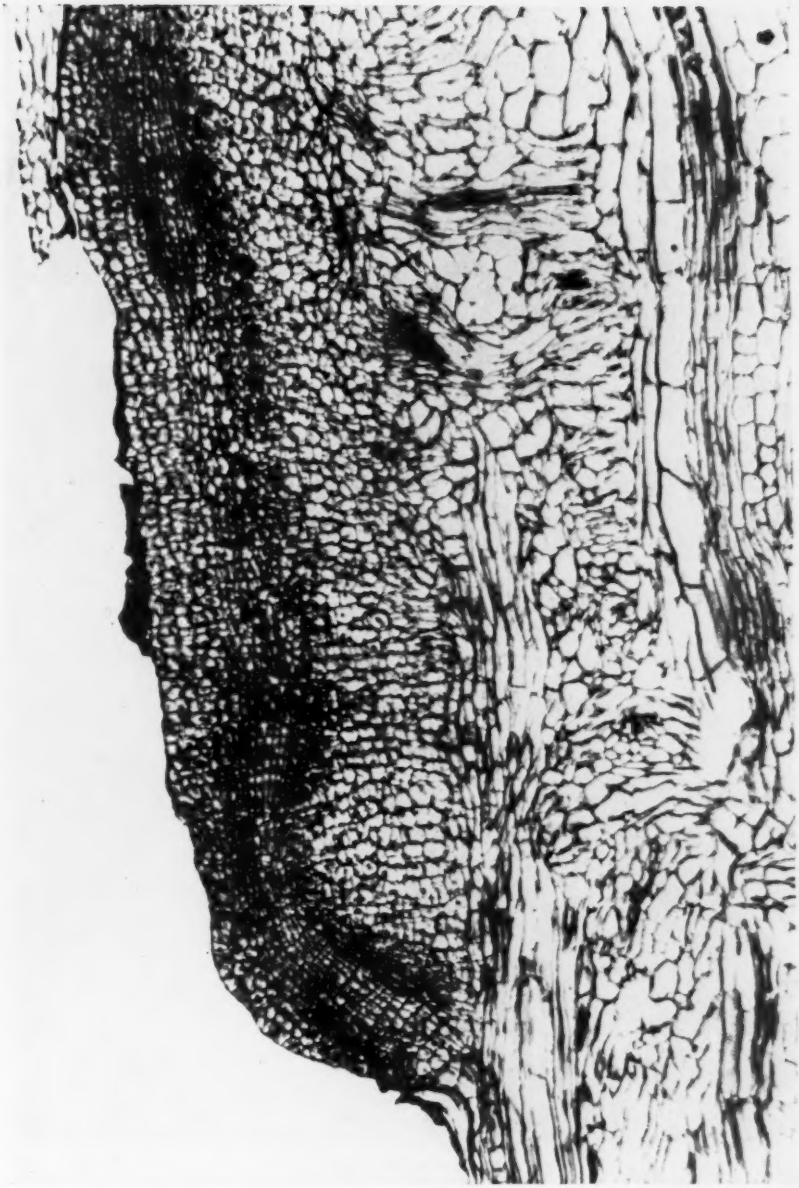


FIG. 9.—Longisection showing in lower portion root primordium with three histogens probably organized before treatment and becoming fasciated (compare with fig. 10*A*). In upper portion three regions of proliferated tissue resulting from treatment. Continuity in organization and similarity of cell types indicate that regions of proliferation correspond with histogens of root. (Longisection of young stem, 7 days after early spray; detail of fourth internode from fig. 3*A*.)

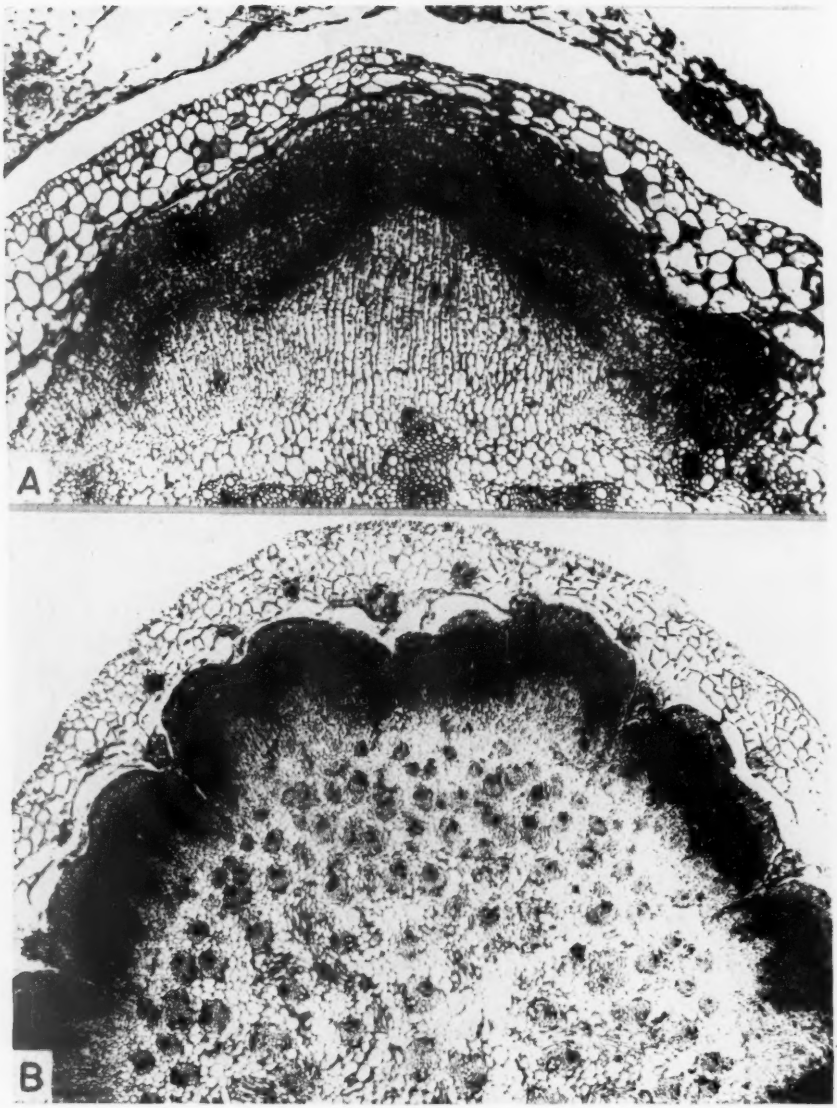


FIG. 10.—*A*, young root primordium becoming fasciated by proliferation of adjoining tissues. *B*, intensely active band of proliferation encircling stem. Indented outline suggesting formation of numerous fasciated root tips as in fig. 1*C*. (*A*, transection in fourth internode of young stem, 11 days after early spray; *B*, transection in fifth internode of young plant, 18 days after early spray. This and subsequent figures from plants grown in the garden.)

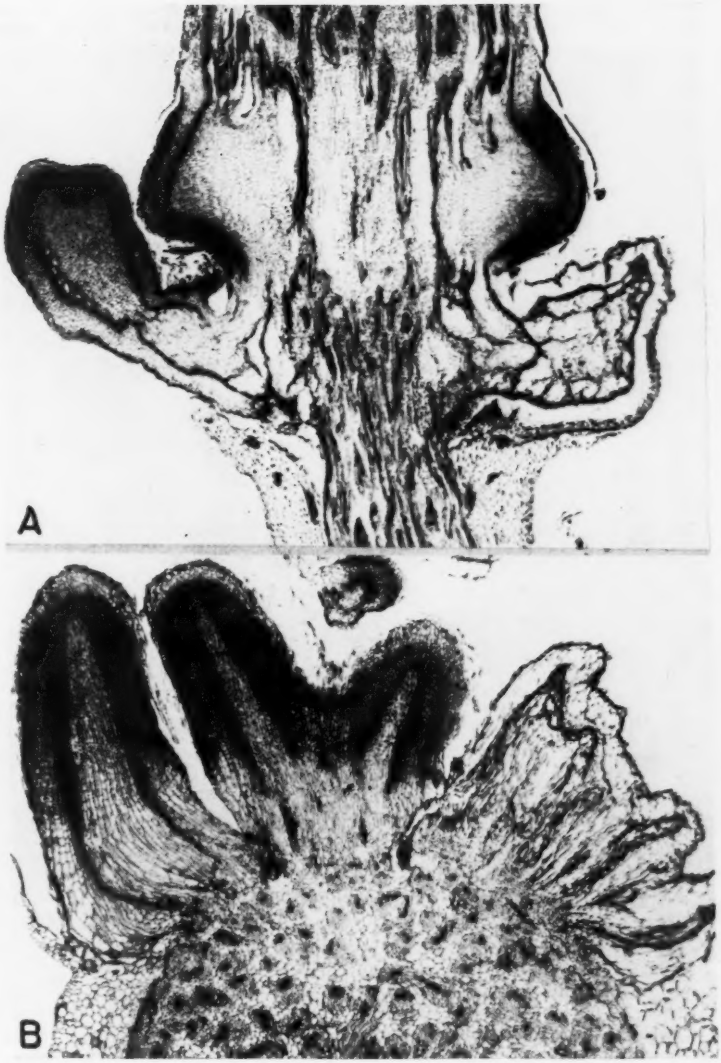


FIG. 11.—*A*, longisection at fourth and fifth nodes showing fasciation of third whorl of roots and formation of heavy ridge of fasciated tissue in place of fourth whorl. (Compare with fig. 1*C*.) Large cells in fasciated root at lower left possibly represent retarded differentiation of vessel segments. *B*, transection in fourth internode showing response in small sector of stem with development of group of fasciated roots, becoming individual roots at their outer tips. (Sections from young plants, 18 days after early spray. *B*, same stem as fig. 10*B*.)

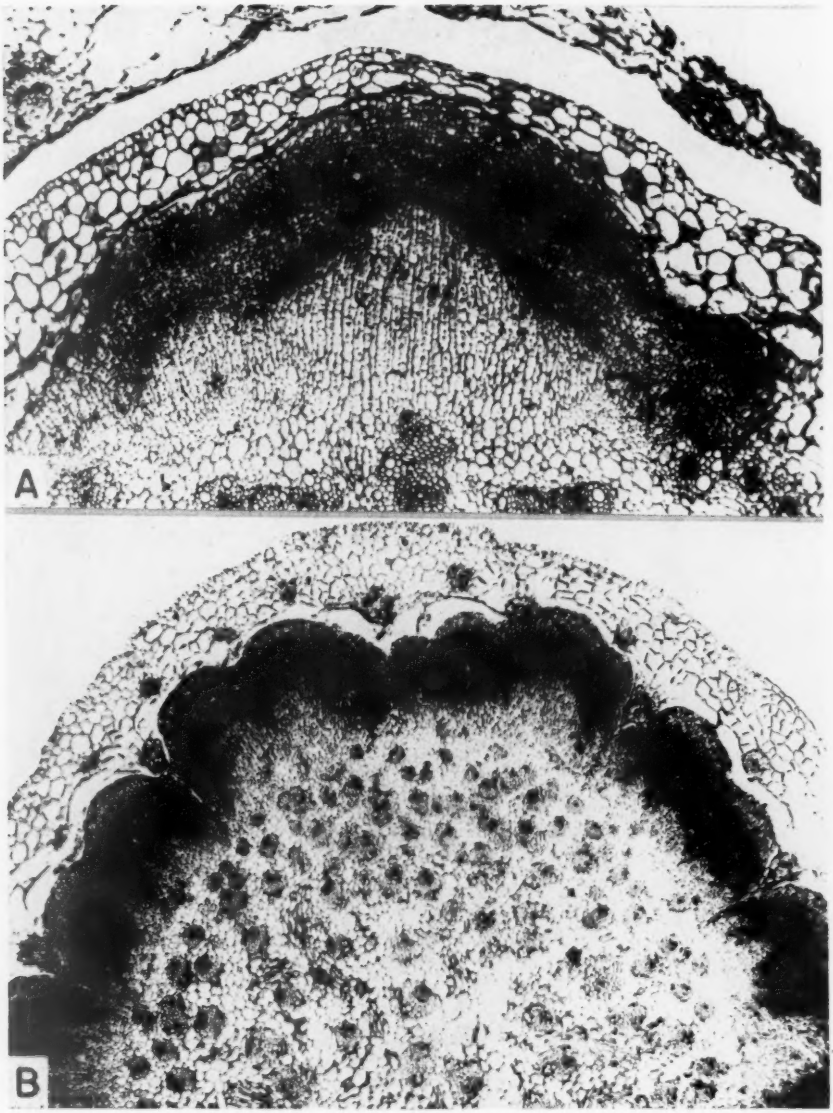


FIG. 10.—*A*, young root primordium becoming fasciated by proliferation of adjoining tissues. *B*, intensely active band of proliferation encircling stem. Indented outline suggesting formation of numerous fasciated root tips as in fig. 1*C*. (*A*, transection in fourth internode of young stem, 11 days after early spray; *B*, transection in fifth internode of young plant, 18 days after early spray. This and subsequent figures from plants grown in the garden.)

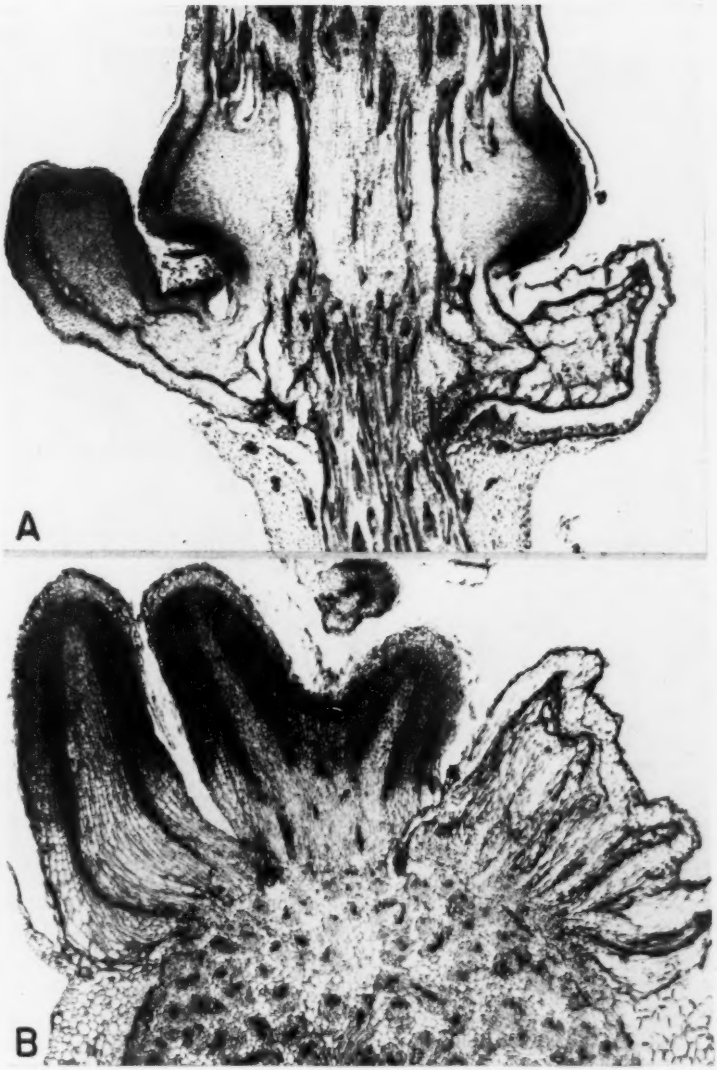


FIG. 11.—*A*, longisection at fourth and fifth nodes showing fasciation of third whorl of roots and formation of heavy ridge of fasciated tissue in place of fourth whorl. (Compare with fig. 1*C*.) Large cells in fasciated root at lower left possibly represent retarded differentiation of vessel segments. *B*, transection in fourth internode showing response in small sector of stem with development of group of fasciated roots, becoming individual roots at their outer tips. (Sections from young plants, 18 days after early spray. *B*, same stem as fig. 10*B*.)

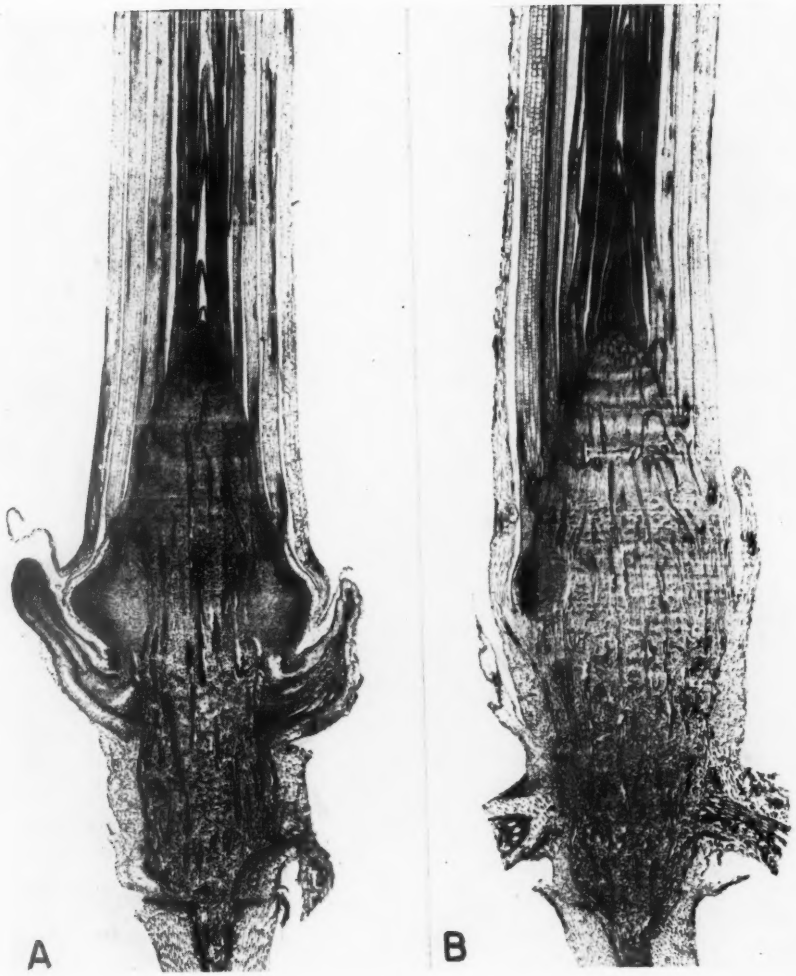


FIG. 12.—Comparison of longisections of young treated plant (*A*), 18 days after early spray, and untreated plant (*B*) of same age. *A*, proliferation resulting in fasciation in third and fourth whorls of adventitious roots. *B*, proliferation absent; four root whorls developed normally, mature roots of second whorl and root primordium in fourth whorl shown in this section. Leaf and stem growth apparently greater in untreated plant.

ous plants (7, 12, 15). The increase in meristematic activity in the monocotyledonous corn plant occurred in the zone of cells which form the peripheral layers of stelar tissues and lie centrad to the cortical parenchyma (fig. 4). HAYWARD (11) has called this the "meristematic zone." It has been described by AVERY (2) as probably pericyclic in origin and consisting of an almost continuous sheath in which some differentiated xylem and phloem occur. SHARMAN (18) followed its development in relation to descending leaf traces. He also noted that adventitious root initials had their origin in this zone. VAN TIEGHEM and DOULIOT (19) ascribed the formation of adventitious roots in the corn entirely to pericyclic tissue. Although this zone cannot be precisely defined as to inner or outer limits, it was clearly the area in which proliferation commenced.

The first cells to show response were the parenchymatous cells lying immediately outside the young vascular bundles and in the rays between these bundles (fig. 6*A*). Adjoining parenchymatous cells rapidly became active, and finally immature vascular cells also participated. Thus early proliferation was soon continuous in the meristematic zone (fig. 7*A*). The outer limit of proliferation was often sharply defined (fig. 6*B*). Cortical cells beyond this limit infrequently responded. The inner face of the active zone had less definition, and parenchymatous cells lying between the meristematic zone and the ring of mature vascular bundles (fig. 4) began to proliferate (figs. 7, 8*A*) progressively inward.

In the early stages of response proliferation appeared to be without precise pattern. By the seventh day, however, the organization of three regions within the proliferated tissue was evident (figs. 7*B*, 8*B*). Uniformity in cell shape and direction of cell division distinguished each region from the others. The inner region was the widest of the three. Successive tangential divisions formed in transection radial series of isodiametric or slightly elongated cells. They were larger in size and less dense than the other proliferated cells. In the middle region tangential divisions likewise resulted in distinct radial series of cells, but these cells were small, flat, and intensely meristematic. This region was narrow. The outer region consisted of intensely meristematic cells, small, and somewhat rounded in shape. The direction of division was here less clear. Continued proliferation gradually broadened these three regions (fig. 8*B*), the inner one to a marked extent and the middle one least. Tissues of the stem or ensheathing leaf bases exterior to the proliferation became crushed or torn.

Comparison of young and old stages of proliferation and examination of areas of proliferation adjoining root primordia disclosed clearly that these three regions correlated with the three histogens found in the root tip of corn: the outer region with the calyptrogen, the middle region with the dermatogen-periblem, the inner region with the plerome. This is demonstrated in figure 9, where the three regions in the lower portion are organized as a root primordium and then merge into three bands of general proliferation in the upper portion. Development in tissues proliferated in response to treatment was massive and lacked the precision of histogens to be found in a normal root primordium from its earliest stages (fig. 5*A*). This difference was most striking between the multi-layered middle zone of proliferation and the corresponding single-layered dermatogen-periblem of the root primordium. The lack of precision appeared to be the consequence of

the diffuse meristematic activity in the initial stages of proliferation and finally produced the massive fasciation of the older stages.

As proliferation continued, changes in cellular character emphasized further the correspondence of the three regions to tissues derived from the three histogens of the root. In the outer region the exterior cells rounded up and became loosely organized like the outer cells of the root cap. The condition of these cells undoubtedly produced the gelatinous character of the outer surfaces of the fasciated root masses. The middle region was maintained as a narrow layer with a symmetrical pattern of cells similar to the cortical layers of a root. The inner region frequently showed elongated cells like those in the central cylinder. In some specimens the formation of large cells (fig. 11*A*) along the border of this region probably represented the initial but retarded differentiation of vessel segments. Although the development of single roots from fasciated masses was not traced, approximations in the proliferated tissues to the precise organization and differentiation of normal root primordia suggested the manner in which conversion from massive fasciation to the formation of individual roots occurred (fig. 11*B*).

Proliferation appeared to be limited in the nodal areas of the stem. There was little or no activity where axillary bud traces or leaf traces diverged through the meristematic zone. In the internode response was more frequent in the lower half. If the entire internode became active, the degree of proliferation was greater in the lower half than in the upper (fig. 3*A*; compare the two sides of the stem). In several specimens it was noted that young root primordia formed the lowest point of proliferation in an internode (figs. 9, 10*A*). Primordia which were somewhat older at the time of treatment appeared to be unaffected and to grow normally even though proliferated tissues extended to their margins. In some plants wide, even bands of proliferated tissue suggested the origin of ridges and massive collars of fasciation (figs. 8, 11*A*, 12*A*; compare with figs. 1, 2). In other plants the band of proliferated tissues became indented in outline and indicated the development of numerous fasciated root tips (fig. 10*B*; compare with fig. 1*C*). Proliferation sometimes encircled the stem, or only small sectors were involved (compare figs. 5*B* and 6*B*) and groups of fasciated roots developed (fig. 11*B*; compare with fig. 2*B*).

The numerous differences in the pattern of histological development made it possible to account for the almost fantastic variation in the degree and external form of fasciation. It was also possible on the basis of serial sections to corroborate the distribution of response in relation to the nodes of the stem and whorls of adventitious roots (table 1) with that already noted from external observations. Of ten young plants examined in detail, eight showed proliferation above the fifth node (fourth whorl of roots). Four displayed activity above the fourth node (third whorl of roots), and two above the sixth node (fifth whorl of roots). In each of four plants, response occurred in two internodes.

Response in the older plants treated with the later spray appeared to be histologically similar to that in the young plants. Proliferation commenced in the meristematic zone (fig. 6*A*) and continued to develop in the manner already described. The response was limited to higher internodes (fig. 3*B*), the sixth to the eighth (table 1), and proliferation was not so extensive as in the early treat-

ment. This is in keeping with greater response of younger or less mature cells or tissues.

Discussion

Application of foliar sprays of the butyl ester of 2,4-D to young corn plants with four leaves expanded resulted in a major disturbance in root formation. Treatment induced proliferations composed essentially of root-forming tissues, but these tissues were so disorganized that fasciation was the usual consequence of development. The degree of proliferation varied from slight stimulation resulting in the production of numerous individual roots to extreme fasciation where masses of tissue gave little indication of functional roots.

The location of the response to treatment was correlated with the specific leaves which received the spray. The nodes above or below which proliferation took place were those nodes from which sprayed leaves diverged. Table 1 summarizes this relationship for both the early spray on young plants with four leaves expanded and the later spray on older plants with seven leaves expanded. The localization of the response in the two different treatments was rather definite. The extensive telemorphic responses to 2,4-D described for dicotyledonous plants (**6**) were absent in the monocotyledonous corn as treated in this experiment. In experiments by Wood *et al.* (**21**) using a radioactive plant-growth regulator as a tracer, translocation in barley plants also appeared to be more limited than in bean plants. The data, however, are not sufficiently applicable to be conclusive on this point.

The age of the plant tissues also appeared to be a factor in the response. Spray applied to the lower, more mature leaves of the older plants resulted in no apparent disturbance in the lower, more mature internodes and their respective root whorls, but spray applied at the same time to the younger leaves at the higher nodes resulted in proliferation and incipient root fasciation in adjoining internodes. It was also noted that root primordia which reached an older stage of development appeared to be unresponsive. Thus young structures are critically more sensitive to 2,4-D than older structures.

Three observations made in this experiment—the major disturbance in root formation in response to treatment with 2,4-D, localization of the response in correlation with the leaves sprayed, and sensitivity of young structures—have certain practical implications. The corn plant is highly susceptible to 2,4-D injury to the permanent root system when foliar sprays are applied to the first through fifth foliage leaves in early stages of growth. Spraying at an older stage of growth, when root formation at the lower nodes was well established, resulted in little or no damage to these roots. During early growth the newly expanded leaves are capable of translocating 2,4-D or its effect to the stem. The internodes adjoining the respective nodes at which these leaves diverge are also sensitive to 2,4-D at this stage, and proliferation and root malformation result. Since the permanent brace roots of the corn are formed at the lower internodes, it is these important roots which are injured by early sprays. Recovery from this effect and final, but retarded, development of unusually numerous roots occurred in some plants. The total growth of the sprayed plants was less than that of the untreated plants. Martin and Hershey (**14**) have stated that the anatomy of the corn plant makes no provision in the roots and very little in the

stems for recovery from stunting effects that occur during the establishment of these structures.

Differences in varietal sensitivity were also considered in this experiment. Gross and histological observations showed that these were differences in degree rather than in the type of response. The two varieties Early Evergreen and Golden Bantam showed the same histological development of proliferation and fasciation. The degree of response, however, varied in the different varieties. In the garden, Spancross appeared to be more sensitive than the other varieties. In the greenhouse, Marcross and Early Evergreen seemed more sensitive than the other three. Within a single variety, however, response varied from plant to plant, and extreme fasciation was observed in some plants of each variety (figs. 1, 2). Varietal sensitivity, therefore, cannot be rated without larger numbers of plants than those used in this experiment.

Responses of the corn plant to 2,4-D resemble responses reported in other monocotyledons to various growth-regulating substances. In two species of *Lilium*, BEAL (5) found that 3% indoleacetic acid applied to decapitated stems induced proliferation in the parenchyma lying centrifugally and laterally to vascular bundles which were generally the outer ones in the stem. This proliferation resulted in differentiation of adventitious roots. BLOCH (8) applied indoleacetic, indolebutyric, indoleproprionic, and naphthoxyacetic acids to various portions of the stem of *Tradescantia*. He found that response differed with the age of the tissue and was closely correlated with the original distribution of meristematic or potentially meristematic cells. Adventitious root formation was induced in both the node and the internode and from peripheral and internal bundles. As in *Lilium* and in corn the primordia originated in tissue lying adjacent to the phloem. BLOCH noted that gross response in *Tradescantia* was comparable to that in dicotyledons but less vigorous and more localized.

In a comparison of the response to 2,4-D in corn with that in dicotyledons, there are some similarities to be noted. Histological response in both occurs as proliferation induced in stem tissues which would otherwise be matured. The tissues which become active lie mainly at the periphery of the stele. In dicotyledons these tissues may be named somewhat precisely and their development traced. In corn this is not possible, yet the similarity of response affords interesting speculation as to the similarity of tissues affected. In both types of plants the proliferated tissue may be root-forming tissue. One marked dissimilarity, as already described for this experiment, was the limitation or localization of the telemorphic effects of 2,4-D in corn as compared with the extensive translocation of telemorphic effects in dicotyledons. This difference, if generally true, might account in part for the selectivity of the herbicide.

Summary

1. Seven varieties of sweet corn were planted in the garden. When the plants were approximately 9 inches tall and showed four leaves expanded, they were sprayed with a 1% concentration of the butyl ester of 2,4-dichlorophenoxyacetic acid. A second and third series with several of the same varieties were grown in the greenhouse. They were similarly sprayed. Two stages of growth were treated: young plants with four leaves expanded as in the garden, and older plants with seven leaves expanded. Gross observation and histological collections

of the three series were made over a period of 5 weeks.

2. As a result of treatment the leaves showed spray injury, and the youngest exposed leaf in the older plants often remained rolled in the bud and checked the growth of inclosed leaves. Bending of the stem at the nodes was noted in both young and older plants. The major effect of treatment was swelling at the nodes, with stimulation and malformation of associated adventitious roots which in the lower internodes normally form the permanent system of brace roots of the corn. In young plants the third, fourth, and fifth whorls of adventitious roots above the fourth, fifth, and sixth nodes, respectively, were affected. Response varied from extreme fasciation to slight fasciation and the formation of numerous roots. In older plants the lower whorls of roots were unaffected, but at higher nodes—the sixth, seventh, and eighth—swelling and incipient root formation occurred (see table 1).

3. Histological study showed that tissues lying in the meristematic zone at the periphery of the stelar area were stimulated to excessive proliferation. In early stages meristematic activity was diffuse, but in later stages three regions became evident. These regions corresponded with tissues derived from the three histogens found in a corn root tip but lacked the precise organization of a primordium. Root fasciation appeared to be a consequence of this lack of organization. Response was histologically similar in the young and older plants, but proliferation occurred in the fourth, fifth, and sixth internodes of young plants and in the sixth, seventh, and eighth internodes of older plants.

4. A comparison of the results in the two treatments leads to the conclusions that (*a*) 2,4-D induces a major disturbance in root formation in the young corn plant, (*b*) the response is localized in correlation with the leaves sprayed, and (*c*) young structures are critically more sensitive than older structures.

5. These three conclusions, determined under the conditions of this experiment, have some practical implication. The corn plant is highly susceptible to 2,4-D injury to the permanent root system when foliar sprays are applied to the first to fifth foliage leaves in early stages of growth. Older plants are less susceptible to this injury.

6. Response in the corn plant is discussed in relation to response in other monocotyledons and in dicotyledons.

DEPARTMENT OF BOTANY
UNIVERSITY OF CHICAGO

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ATYPICAL GROWTH OF PLANTS. I. CULTIVATION OF VIRUS TUMORS OF RUMEX ON NUTRIENT AGAR[1]

PAUL R. BURKHOLDER AND L. G. NICKELL

Introduction

Tumors which are formed in sorrel, *Rumex acetosa* L., following the establishment of the virus *Aureogenus magnivena* Black in these plants, have been isolated from roots and grown in pure culture (1, 2, 3). These tissues carried through an extensive series of subcultures are capable of transmitting the virus through graft unions to healthy plants, which in turn develop tumors. Both normal and atypical tissues of *Rumex* lend themselves to cultivation in chemically defined media in pure culture and, therefore, offer decided advantages as material for the study of fundamental properties which must underlie the marked and obvious changes in structure which characterize tumefaction. Elucidation of the mechanisms by which normal plant cells are transformed into tumors, how these atypical masses of disorganized tissues are propagated autonomously, and the nature of the reproducing agents which are capable of infecting other susceptible normal plants constitute important problems in this field. DE ROPP (7) has recently summarized some of the properties evidenced alike by plant and animal tumors with special reference to etiological agents, such as viruses, hormones, and genetic factors. On the grounds of comparative biochemistry, an increasing knowledge concerning tumefaction in plants should contribute substantially to a comprehension and mastery of neoplastic transformations.

Preliminary to studies on these problems, it becomes important to grow the plant tumors satisfactorily in simple media of known composition. BLACK (3)

[1] This work has been aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

found that the root tumors of sorrel grown on WHITE's medium doubled in size during a period of 3 weeks. Other types of disorganized or relatively undifferentiated tissues, such as crown galls, *Nicotiana* hybrid tissue, auxin-induced callus, and proliferating embryos, have been produced and studied extensively by BRAUN and WHITE (4), WHITE (14), RIKER (11), GAUTHERET (9), DE ROPP (6), and CURTIS and NICHOL (5). Our efforts have been directed at first toward the development of a satisfactory medium for production of large amounts of material of virus wound tumor in chemically defined media and, in the second place, toward a comparison of the nutrition and growth habits of virus wound tumor with the behavior of other kinds of atypical plant growths.

Material and methods

The tumor tissue used was obtained through the kindness of Dr. L. M. BLACK (3). It bears the strain designation R_1 and was isolated by him from *Rumex acetosa* root tumors in September, 1944. In our experiments the tumors were cultivated on nutrient agar slants in Pyrex test tubes (25 × 150 mm.) plugged with cotton. Difco Noble agar was used at 0.8–1.0% to provide a gel substrate. Sucrose was employed as a carbon source generally added at levels of 1 or 2% to the various inorganic solutions. Water for media was twice distilled in Pyrex glass, and the pH value, measured with a glass electrode, was adjusted with NaOH or HCl where necessary. The prepared media were sterilized by autoclaving at 15 pounds pressure for 15 minutes. The cooled agar slants were inoculated aseptically with fragments of tumor tissue carefully cut with a sterile knife. The knives for cutting and transfer were made of triangular pieces of razor blade spot-welded to sewing needles and fixed into bacteriological needle-holders. All cultures were allowed to grow under fluorescent lights at 500 foot-candles in a room with the temperature controlled at 23°.5 C.

Several stock solutions were used for convenience in making the various experimental media. These stock solutions are the following: (*a*) Solutions of individual salts each at a concentration of 0.1 *M*—KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$, $CaCl_2$, KCl, and $MgCl_2$. Ten milliliters of each solution were used for each millimole (mM) of required salt per liter of final medium. (*b*) Vitamin solution—thiamin HCl, 0.001 gm.; pyridoxin HCl, 0.008 gm.; nicotinic amide, 0.008 gm.; in 100 ml. distilled water. This solution was used at the rate of 10 ml. per liter of nutrient. (*c*) Trace-elements solution containing in a liter the following: H_3BO_3, 570 mgm.; $MnCl_2 \cdot H_2O$, 360 mgm.; $ZnCl_2$, 625 mgm.; $CuCl_2 \cdot 2H_2O$, 268 mgm.; $Na_2MoO_4 \cdot 2H_2O$, 252 mgm.; and $Fe_2(C_4H_4O_6)_3$, 1825 mgm. One milliliter of this stock solution per liter of final medium provided the following added amounts of the trace elements in p.p.m.: B, 0.1; Mn, 0.1; Zn, 0.3; Cu, 0.1; Mo, 0.1; and Fe, 0.5. The stock solutions of salts were kept in glass-stoppered Pyrex bottles at room temperature, and the solutions of vitamins and trace elements were stored under benzene in bottles kept in a refrigerator at 5° C.

Experimentation

VARIATION OF MEDIA.—Because of the desirability of comparing the growth of the virus wound tumor with that of other plant tumors and for the purpose of increasing the stock, the original cultures growing on WHITE's medium were transferred to a similar medium as modified by RIKER (11). Preliminary experiments

were then designed for the purpose of determining the responses of the tissue to variations of nitrate and phosphate in the media. Several nutrient media, based on the work of RIKER with cultures of sunflower callus, were employed as follows: RIKER's medium contained 0.016 *M* nitrate and 0.001 *M* phosphate; the same medium but with the phosphate adjusted to one-fifth and to five times this level; the basal medium with nitrate varied at one-fifth and five times the original level. The pH value was adjusted to 5.8. After a period of 3 weeks, it was found that only fair growth occurred in medium containing 0.001 *M* phosphate, and still less growth took place in the lower concentration, whereas satisfactory growth appeared in the nutrient with 0.005 *M* phosphate. On the contrary, it was found that 0.016 *M* nitrate allowed only fair growth, that lowering the concentration resulted in better growth, and that increasing the concentration caused the tissue to turn dark brown with little obvious growth. These preliminary results suggested the range within which to work and provided useful information for the design of further experiments.

NUTRIENT TRIANGLE EXPERIMENTS.—By the use of the triangle system, media were then formulated for the purpose of obtaining a more accurate idea concerning the growth responses of the tumor tissue to varied proportions of the major nutrient elements. The chief obstacle in reaching a final decision by this method lies in the fact that, as one ion is varied, the others in the triangle are also varied. By using the information gained in such triangle experiments, however, the "best" medium of this group (no. 19, table 2) was selected as basal, and a new experiment was next designed in which the concentration of one ion at a time was varied (table 2) over a considerable range. This led finally to the choice of an excellent medium which permits the production of tumor tissue more rapidly than in any previous work.

These triangle experiments were set up essentially according to the traditional methods used recently by VOTH (13) and by RIKER and associates (10, 11). The number of proportional parts of cations or anions was set at ten for each side of the triangle, and the fraction of each ion in a given treatment was adjusted in tenths of the total, according to the diagram in figure 1. The various media were made up as described under "material and methods." The initial pH value of all these treatments was 5.8. In the first set of experiments the cations K, Mg, and Ca were kept in the proportions of 8:1:1. In the second set the proportions were 4:3:3. The three anions, nitrate, phosphate, and sulfate, were varied as shown in table 1 and figure 1. Stock cultures to be used in inocula were grown on RIKER's nitrate medium with the concentration of nitrate reduced to one-half that employed for sunflower callus. The tumors were cut into desired sizes, weighed aseptically in small covered dishes, and transferred to the various media. All media used in this and in subsequent experiments were set up in replicates of ten. After a growing period of 4 weeks fresh weights were determined, and the growth value was calculated by dividing the final weight by the initial inoculum weight.

Very healthy and rapidly growing tissues of this tumor are light green in color with no trace of brown pigment. Spectrophotometric determinations on extracts from these tumors showed the presence of chlorophylls *a* and *b*. It was found that changes of color are closely correlated with changes in growth value (tables 1, 2). The higher the growth value, the higher the ratio of green to brown. Thus,

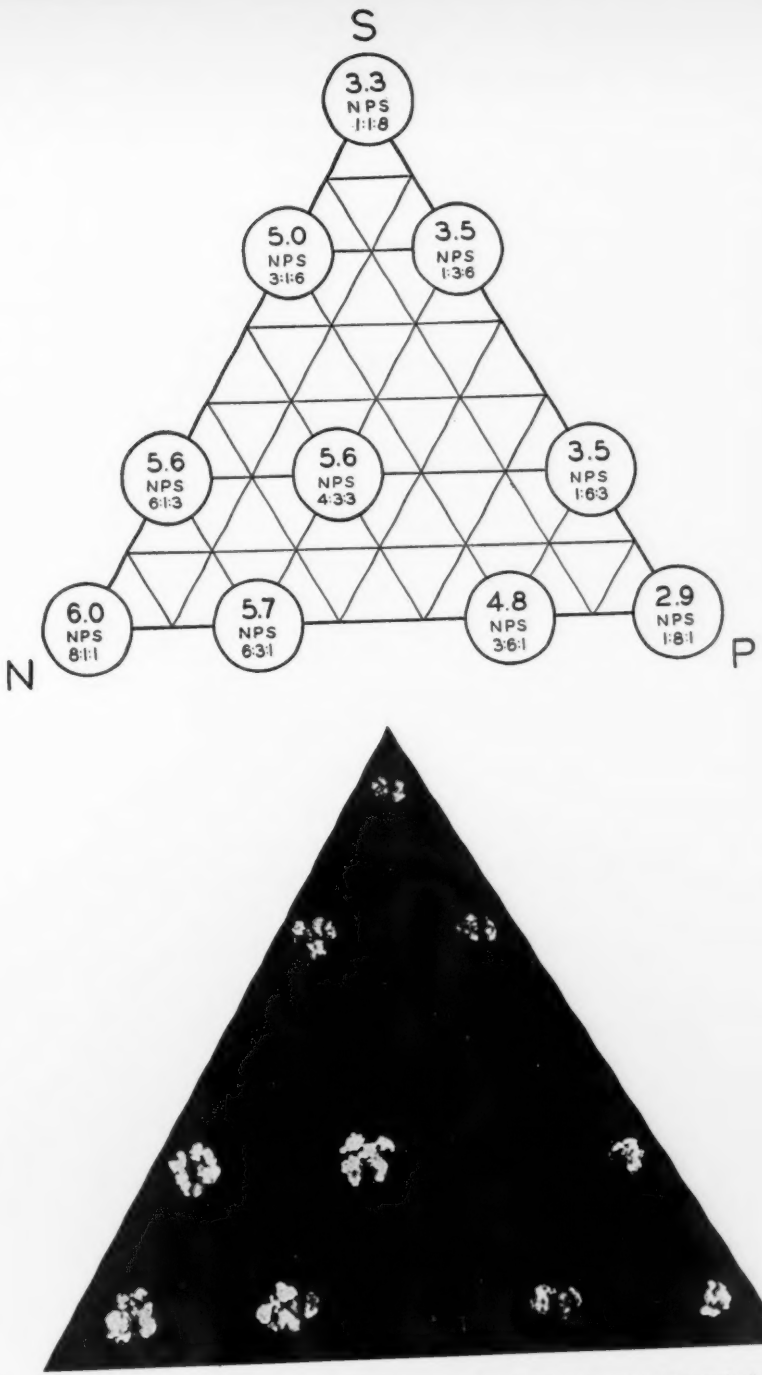

FIG. 1.—Anion triangle (*above*) showing proportions of N:P:S (millimoles per liter) in typical experiment with ten treatments. Large numbers in circles represent average growth values obtained with ten replicated cultures for each of ten treatments. Photograph (*below*) shows appearance of tumors from single representative cultures at each position on triangle after growth period of 4 weeks.

TABLE 1

GROWTH RESPONSES OF *Rumex* TUMORS IN NUTRIENT AGAR CONTAINING SUCROSE WITH VARIED PROPORTIONS OF N, P, AND S IN A TRIANGLE SYSTEM. (ALL PROPORTIONS OF NUTRIENT IONS EXPRESSED IN MILLIMOLES)

Medium no.	Proportions of			Relative color		(*a*) Inoculum weight in mgm.	(*b*) Final weight in mgm.	(*b*)/(*a*) = growth value
	NO_3	PO_4	SO_4	Green	Brown			
	K, Mg, and Ca in proportions 8:1:1							
1	8	1	1	5	1	52.1	312.1	6.00
2	6	3	1	4	1	68.2	386.0	5.66
3	6	1	3	4	2	53.5	298.4	5.58
4	4	3	3	3	2	63.3	357.4	5.64
5	3	6	1	2	2	53.1	256.1	4.82
6	3	1	6	2	2	45.6	228.6	5.01
7	1	8	1	1	3	52.9	154.9	2.92
8	1	6	3	1	3	41.7	144.9	3.47
9	1	3	6	1	3	52.6	182.7	3.47
10	1	1	8	1	4	38.0	126.4	3.33
	K, Mg, and Ca in proportions 4:3:3							
11	8	1	1	4	1	34.0	214.7	6.31
12	6	3	1	3	1	41.6	189.2	4.55
13	6	1	3	3	1	33.6	205.3	6.11
14	4	3	3	3	1	34.3	289.0	8.42
15	3	1	6	2	2	41.0	149.8	3.65
16	1	6	3	1	3	48.0	145.5	3.03
17	1	3	6	1	3	49.3	138.0	2.80
18	1	1	8	1	4	54.5	143.0	2.62

TABLE 2

GROWTH RESPONSES OF *Rumex* TUMORS IN NUTRIENT AGAR CONTAINING SUCROSE WITH K, CA, AND MG IN PROPORTIONS OF 12:6:2 AND NO_3, PO_4, AND SO_4 VARIED INDEPENDENTLY AS INDICATED. (ALL PROPORTIONS OF NUTRIENT IONS EXPRESSED IN MILLIMOLES)

Medium no.	Proportions of			Relative color		(*a*) Inoculum weight in mgm.	(*b*) Final weight in mgm.	(*b*)/(*a*) = growth value
	NO_3	PO_4	SO_4	Green	Brown			
19	8	8	4	5	1	54.0	325.0	6.02
20	8	4	4	4	2	36.6	194.4	5.31
21	8	2	4	3	3	26.6	111.1	4.18
22	8	1	4	2	4	28.5	81.5	2.86
23	8	8	2	6	0	36.7	331.5	9.03
24	8	8	1	6	0	34.0	303.3	8.92
25	8	8	0.5	6	0	31.3	303.3	9.69
26	4	8	4	6	0	35.3	235.0	6.65
27	2	8	4	4	2	36.5	170.0	4.68
28	1	8	4	2	3	34.1	144.4	4.23
29	4	4	2	4	2	49.5	184.8	3.73
30	4	2	2	3	2	37.3	178.0	4.77
31	4	1	2	3	3	38.0	148.0	3.89
32	4	0.5	2	2	4	30.3	119.0	3.92
33	4	4	1	5	1	35.0	237.5	6.78
34	4	4	0.5	5	1	35.8	139.0	3.88
35	4	4	0.25	5	1	36.9	134.2	3.64
36	2	4	2	4	2	41.6	155.5	3.74
37	1	4	2	1	4	50.4	164.5	3.26
38	0.5	4	2	1	4	39.3	102.0	2.59

coloration provides an additional criterion for evaluating the effectiveness of a medium for these tumors.

VARIATION OF ANIONS INDEPENDENTLY.—With medium no. 19 as a starting point, each of the anions, NO_3, PO_4, and SO_4, was adjusted one at a time at levels of ×1, ×1/2, ×1/4, and ×1/8 the concentration used in the basal medium. All the media prepared from stock solutions were adjusted to pH 5.8, sterilized, and inoculated with weighed pieces of tissue. After a growing period of 4 weeks under controlled conditions, the growth values and relative colors of these cultures were determined. The results are shown in table 2 and in the graphs in figure 2. A marked reduction of the growth value was associated with decrease in phosphate below 8 mM. A decrease in nitrate below 8 mM resulted in diminished growth values similar to those with lowered phosphate, but not so marked. Concentrations of sulfate used below 2 mM permitted excellent growth, but at a level of 4 mM growth was not so good. Media nos. 23, 24, and 25 all supported good growth. Media nos. 29–38 were made to duplicate media nos. 19–28, except that the concentration of each of the inorganic ions was reduced to one-half. The results shown in table 2 indicate an obvious lowering of growth values in media with reduced ion concentrations, suggesting that the presence of nutrient ions in higher concentration is essential for near optimal growth.

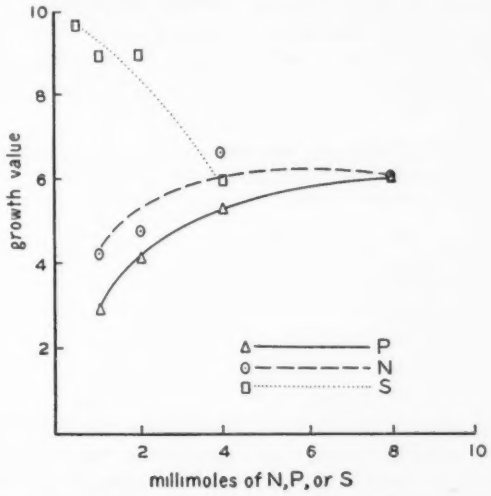

FIG. 2.—Growth values of tumors plotted against millimoles of N, P, and S per liter of nutrient agar

EFFECT OF Na^+ AND Cl^- IONS.—In the various media discussed above the addition of Na^+ and Cl^- ions was at times necessary. It was deemed advisable, therefore, to set up a series of cultures supplied with increasing amounts of NaCl in order to find whether these ions have any inhibitory or stimulating effects on growth. A solution which contained neither Na^+ nor Cl^- ions was used as the basal medium (no. 3 of table 1). The levels of NaCl added to this basal solution varied over a range from 0 to 10 mM. Inoculations were made as in previous experiments, and, after a grow-

ing period of 3 weeks, growth values were determined. Slight stimulation occurred at levels of 2 and 5 mM of NaCl. No effect was observed at 7 mM. Slight growth inhibition was found only at the highest level of NaCl (10 mM), which was greater than the amounts of these ions present in any of the media previously employed.

INORGANIC NITROGEN SOURCES.—It seemed desirable to make a simple comparison of the ability of the tumor tissue to utilize different forms of inorganic nitrogen. With medium no. 24 as a basis, and the pH value adjusted to 5.8, nitrogen sources were supplied in the form of only nitrate, only ammonium, and as ammonium nitrate. As shown in table 2, growth on nitrate at a level of 8 mM was excellent. When NH_4NO_3 was substituted for nitrate (medium 43, table 3), the growth value was lower. When only the ammonium ion was present as a nitrogen source (medium 44, table 3), the growth value was still lower. The results which have been obtained thus far are in general agreement with the data published by RIKER (11) for sunflower callus, in that nitrate is an excellent source of nitrogen for the cultivation of atypical tissues. The influence of other factors, such as pH value and carbohydrate metabolism, upon the utilization of oxidized and reduced nitrogen by these tumors remains to be investigated.

TABLE 3

GROWTH RESPONSES OF *Rumex* TUMORS ON MEDIUM NO. 24 AND MODIFICATIONS SUBSTITUTING NH_4NO_3 AND NH_4^+ AS NITROGEN SOURCES

MEDIUM NO.	mM		RELATIVE COLOR		(a)	(b)	(b)/(a)
	NO_3	NH_4	Green	Brown			
24	8	0	6	0	61.5	396.0	6.44
43	4	4	5	1	73.0	419.3	5.74
44	0	8	5	1	70.5	357.8	5.00

VARIATIONS IN SUCROSE AND HYDROGEN-ION CONCENTRATION.—It seemed desirable to determine the influence of varied levels of sugar and pH upon growth of the tumor tissues in a medium containing satisfactory proportions of salts (no. 24). An experiment was designed with sucrose varied over a range from 0 to 100 gm./l. The resulting growth values, determined after 3 weeks, are presented in figure 3. Growth is evidently proportional to sucrose concentration up to about 2%. Satisfactorily high growth values appear in the range of 1–3%, after which growth is less with increasing concentrations. Brown pigmentation was noted in cultures on media with 0, 0.25, and 10.0% sucrose, but a green, healthy appearance was observed in the others.

The influence of pH value of the medium (no. 24) was studied by adjusting the reaction with added NaOH or HCl to cover the range pH 3.15–8.45. After a period of 3 weeks, the final pH value was measured and growth values determined (table 4). Relatively high growth values were obtained over the range pH 3.4–6.2. The pH values at the end of the growing period showed rises in the initial range of 3.4–5.3 and a decrease below and above this range. A lowering of the reaction generally in the range unsatisfactory for growth, and a rise in the range satisfactory for growth, are to be noted. Prefer-

ential absorption of NO_3 may offer an explanation for rise in pH in media supporting rapid growth of the tumors, in a manner which has been described by TRELEASE and TRELEASE (12) for other plant material.

When this experiment was repeated, similar data were obtained, with somewhat better growth evidenced in the lower range of pH values. The results of the second experiment (fig. 4) suggest a trimodal curve for growth over the wide range of pH values. The pattern of this growth curve may be related to the isoelectric points of important protein constituents of the tissues.

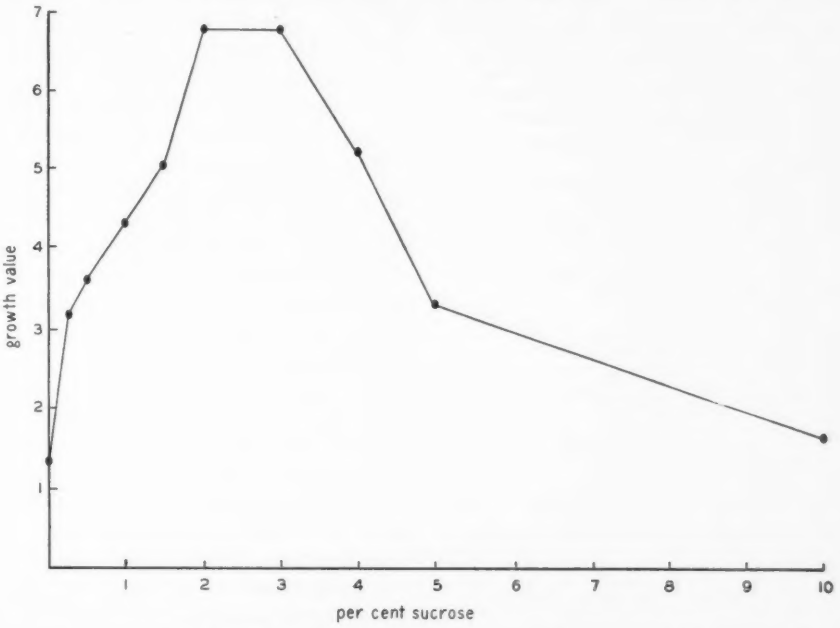

FIG. 3.—Growth values of tumors produced in nutrient agar with sucrose varied from 0 to 10%

PREPARATION OF A SATISFACTORY MEDIUM (NO. 24).—Of all the media studied, no. 24 may be taken as being most satisfactory for rapid growth and maintenance of green living tissue. Because of the softening of the agar which occurs during heat sterilization of this medium, it is recommended that the agar and the nutrient solution be sterilized separately and pipetted together into test tubes. To make a liter of this medium, 500 ml. of 2% agar in distilled water are brought to the boiling point and then dispensed into test tubes (5 ml. per tube), plugged, and autoclaved. This liquid agar is kept warm until the nutrient can be added and mixed with it. The following nutrient solution suitable for this purpose is made from stock solutions and C.P. sucrose:

20 ml. 0.1 *M* KNO_3	10 ml. 0.1 *M* $MgCl_2$
30 ml. 0.1 *M* $Ca(NO_3)_2$	10 ml. vitamin solution
80 ml. 0.1 *M* KH_2PO_4	1 ml. trace-elements solution
10 ml. 0.1 *M* $MgSO_4$	
30 ml. 0.1 *M* $CaCl_2$	20 gm. sucrose
20 ml. 0.1 *M* KCl	289 ml. water

The pH value is adjusted to about 5.2, and the solution is sterilized by autoclaving. An equal volume of nutrient solution is then pipetted aseptically into the tubes containing the hot sterile agar. The tubes are shaken slightly to mix and allowed to cool in a slanted position. Both the agar and the nutrient solution are made up at twice the final required concentration, and the 50–50 mixture provides a highly satisfactory nutrient agar. The final concentrations of the constituents of this medium are: KNO_3, 0.002 *M*; $Ca(NO_3)_2$, 0.003 *M*; KH_2PO_4, 0.008 *M*; $MgSO_4$, 0.001 *M*; $CaCl_2$, 0.003 *M*; KCl, 0.002 *M*; $MgCl_2$, 0.001 *M*; sucrose, 2%; thiamin, 100 μgm./l.; pyridoxin, 800 μgm./l.; niacinamide, 800 μgm./l.; agar 1%; and specially added trace elements (in p.p.m.)—B, 0.1; Mn, 0.1; Zn, 0.3; Cu, 0.1; Mo, 0.1; and Fe, 0.5.

TABLE 4

GROWTH RESPONSES OF VIRUS TUMORS OF *Rumex* TO VARIATIONS OF pH IN BASAL MEDIUM CONTAINING ESSENTIAL SALTS, NO_3, AND 2% SUCROSE

Initial pH	Final pH	Relative color		Growth value
		Green	Brown	
3.1	2.0	0	2	0.78
3.4	4.6	6	0	6.72
4.0	4.8	6	0	6.41
4.7	4.8	6	0	7.00
5.0	5.2	6	0	7.72
5.2	5.0	6	0	9.37
5.5	5.1	5	0	5.80
5.8	5.2	5	0	6.57
6.0	5.2	4	1	6.45
6.2	5.3	5	1	8.22
6.6	5.5	2	2	3.21
6.8	5.7	1	3	3.01
7.5	6.5	1	2	2.56
8.0	6.8	0	3	2.36
8.4	6.9	0	4	1.70

DESCRIPTION OF TUMORS.—Virus wound tumors of *Rumex acetosa* growing on a favorable nutrient gel in the presence of light consist of irregular light-green masses of tissue characterized by indefinite form and loosely cohering meristematic nodules which are readily separated and capable of indefinite growth (fig. 5*A*). Increase of cellular material can proceed with relative rapidity so that growth values of about 7–9 are obtained in 3 weeks. These cultures can be kept without transfer for several months. The tumors are characterized by numerous zones of proliferating cells located generally at the periphery of the nodular masses (fig. 5*B*, *C*). Differentiation of vascular elements occurs eventually in each growing zone, but there is an obvious lack of cell and tissue organization in any normal fashion. In some of the growing nodules, which consist mainly of parenchymatous cells, there is a suggestion of the formation of vascular bundles (fig. 5*D*) with seriate groups of cells arranged as if derived from a cambium (fig. 5*E*). Numerous crystalline inclusions, presumably calcium oxalate, are apparent by microscopic examination of dissected tumors. In general, the superficial appearance of these virus tumors is similar to that of other disorganized plant growths. All plant tumors lack the common taxonomic and morphological characteristics of the species from which they were derived and resemble one another in general appearance. Some of these disorganized growths, as, for example, *Nicotiana* hybrid tissue, carrot callus, orchid embryo callus, etc., apparently retain certain genetic properties which, under proper conditions, permit occasional expression of differentiation in the direction of forming typical organs. Among the thousands of cultures of *Rumex* tumors thus far observed in our laboratory, however, no development of root, stem, or leaf has ever occurred. Close similarity exists between the crown

gall and virus wound tumor with respect to the disorganized arrangement of the cellular masses comprising these tumors, as well as the restricted manner in which tendencies toward differentiation are evidenced (15). Both types of tumor are capable of bringing about transformation of normal cells of the same species to plants which then developed enlarged veins and typical root tumors. The transmission of an agent from sick to healthy plants through agallian leaf hoppers, as shown by BLACK (1), offers further evidence for a virus etiology. Our attempts thus far to concentrate the virus from tumor cultures and study it with the

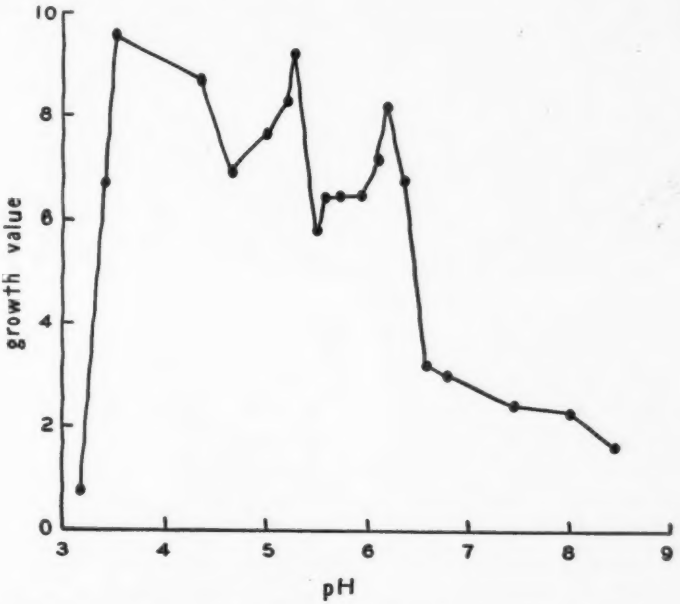

FIG. 4.—Growth values of tumors cultivated in satisfactory medium (no. 24) with initial pH value varied from 3.1 to 8.5.

form atypical growths (6, 8), and both appear to lack the ability in themselves to become organized into organs and tissues characteristic or the normal species from which the tumors were derived.

The *Rumex* tumor contains a virus-like agent which Dr. BLACK demonstrated to be capable of inducing systemic infection in normal plants following grafting of the tumors back into healthy individuals. We have been successful in grafting the tumors into leaves of healthy *Rumex* electron microscope have failed. Moreover, we have been unable to detect the presence of virus antigen in these *Rumex* tumors by serological means using saline extracts injected into rabbits. The growth responses of the wound tumors to relatively high concentrations of phosphate and nitrate appear to be in accord with a concept involving the multiplication of virus-like nucleoprotein in the infected tissues. The feasibility of growing large amounts of virus-containing tumors

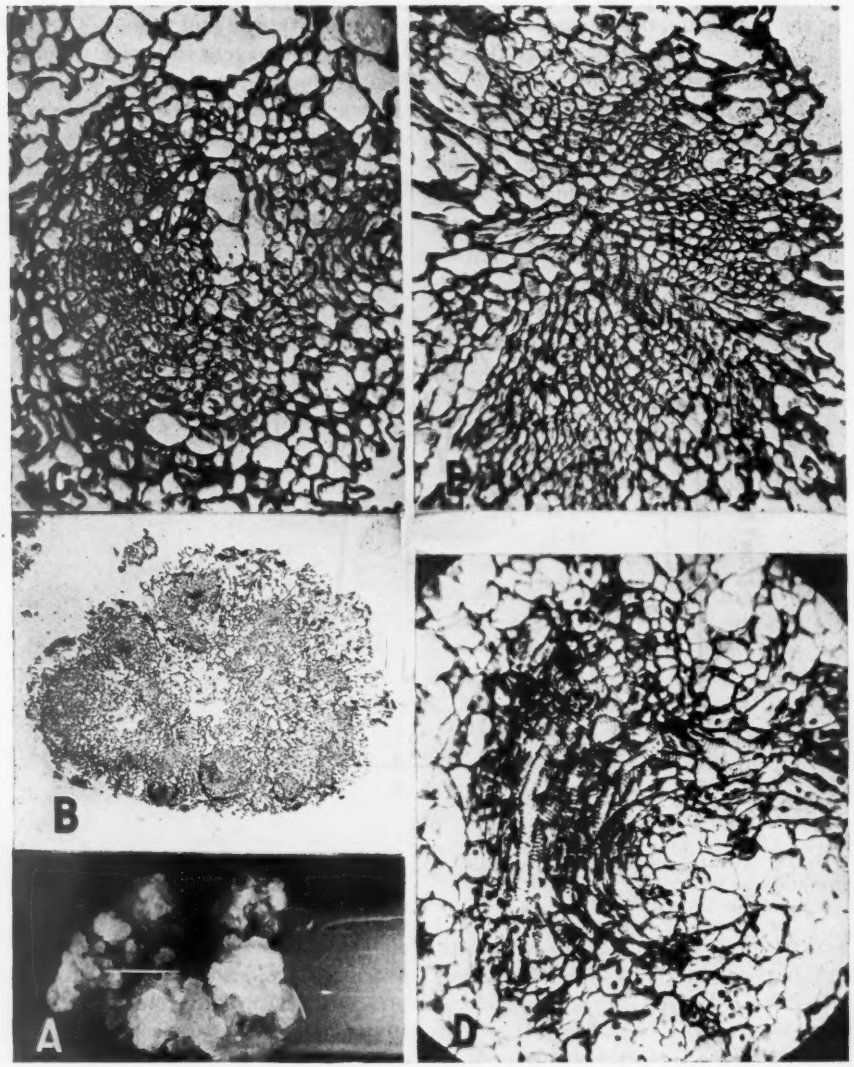

FIG. 5.—*Rumex* virus wound tumor. *A*, appearance of culture on agar slant (×1.33). *B*, section through small portion of tumor showing parenchymatous ground structure with peripheral meristematic or bundle zones (×15). *C*, section of meristematic zone consisting of relatively small nucleated cells with some vascular elements and accompanying vacuolated parenchyma of ground tissue (×110). *D*, section showing pronounced differentiation of scalariform tracheids (×110). *E*, seriate arrangement of cells together with vascular differentiation suggests formation of vascular bundle (×110).

on a simple basal medium of known constituents offers distinct advantages for further investigation of the many problems which can be found in the fields of virology, morphogenesis, and oncology.

Summary

1. Virus wound tumors of *Rumex acetosa* were cultivated satisfactorily in a chemically defined medium containing inorganic salts, sucrose, B vitamins, and agar. Best growth was obtained in media with relatively high concentrations of nitrate and phosphate, sucrose 2–3%, agar 1%, and with the pH value adjusted to about 5.2.

2. The tumor consists of small parenchymatous nodules, containing meristematic zones and some differentiated elements, loosely held together to form a disorganized mass which has never been observed to differentiate normal plant organs.

3. When the tumor was grafted onto leaves of healthy *Rumex* plants, enlarged veins and tumefaction of the roots resulted.

4. This virus-infected tumor tissue, showing rapid growth and having apparent stability in a simple basal medium, provides valuable experimental material for further study of problems in the fields of virology and growth and development.

OSBORN BOTANICAL LABORATORY
YALE UNIVERSITY

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RELATION OF OXIDATION-REDUCTION POTENTIAL, RESPIRATION, AND CATALASE ACTIVITY TO INDUCTION OF REPRODUCTIVE DEVELOPMENT IN SUGAR BEETS

MYRON STOUT[1]

Introduction

The internal factors responsible for reproductive development in plants have long been a subject of interest and speculation. Although much has been learned about the role of environmental factors in stimulating the change from vegetative to reproductive development, relatively little has been discovered concerning the internal factors involved. The purpose of the present paper is to report measurements of several internal factors that are apparently associated with induction of flowering in sugar beets and to point out that they are correlated with changes in the oxidation-reduction balance of certain regions of the plant.

The possibility that induction of reproductive development may be correlated with such changes in the oxidation-reduction balance was suggested by several considerations. The nature of the environmental factors, temperature and light, suggests a change in energy values, and these are usually associated with oxidation and reduction processes in plants. The evident relationship of these factors to energy changes and plant processes will be discussed more fully in a later section of this paper. The fact that the reproductive stimulus can be translocated through grafts of beets (18) and other plants (3, 11, 15) or even very tightly bound diffusion contacts (10) but that apparently no such stimulating substance, or substances, has been extracted and effectively introduced into another plant suggests that the substance, or substances, involved is very labile to atmospheric oxidation when outside of plant tissue. AIROLA and CHRIST (2) reported a decrease in the oxidation-reduction potential of the tissue fluid of cabbage as a result of "winter hardening" (exposure to cool temperatures).

Rate of respiration was also thought of as a factor possibly associated with induction of flowering and a change in oxidation-reduction potential, since respiration is an oxidation process. MURNEEK (17) showed that increased respiration is correlated with photoperiodic induction of flowering in Biloxi soybean, a short-day plant. There is an increase in rate of respiration subsequent to certain chemical treatments which have been found to favor flowering. COOPER and REECE (7) demonstrated that flowering could be induced in pineapple plants by subjecting them to low concentrations of ethylene or acetylene. FOSTER (9) reported that a species of *Aechmea* was induced to flower prematurely by exposure to 0.1% ethylene for 24 hours. The cool-temperature requirement for flowering in certain deciduous orchard trees has been modified by several kinds of oil-chemical spray treatments (4).

Measurement of catalase activity in beet tissue was suggested by the work of others with other species. KNOTT (13) reported that catalase activity in the apical buds of spinach plants which were pro-

[1] Physiologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Agricultural Research Administration, U.S. Department of Agriculture.

ducing seedstalks was lower than that in the apical buds of vegetative plants. HIBBARD (12) found that catalase activity in the tip of Biloxi soybean was reduced when placed under the short-day conditions favorable for reproduction. He stated that there was a slight increase in activity after about the twentieth day of exposure to short photoperiods but that the activity level in plants on short day always remained lower than in plants on long photoperiods.

The sugar beet is well suited to the study of internal factors involved in reproductive development. One reason for this is that the ordinary commercial biennial varieties differ from annual types that are available for experimental purposes in regard to environmental factors required for floral induction. The biennials require prolonged cool-temperature exposure (thermal induction) followed by, or concurrent with, exposure to long days or long light periods (photoperiodic induction). The annuals, although affected to some extent by thermal induction, appear for all practical purposes to require only photoperiodic induction. By means of grafts of annuals and biennials it has been shown (18) that an annual scion or stock given long-day exposures will transmit to a biennial beet grafted with it and kept in darkness a stimulus that will induce bolting in the biennial even though the latter has not been exposed to cool temperatures. If an annual is grafted with a biennial, and the annual part is placed in darkness while the biennial part is given long-day light exposures, bolting is not induced in the annual. If, however, the biennial has been exposed to cool temperatures so that it bolts, it then transmits something to the annual that causes the annual to bolt. From these facts it can be seen that it is feasible to compare internal changes due to the combined action of light and temperature with changes due to either.

A second favorable fact is that biennial beets which have received adequate thermal induction can be completely reversed to the vegetative condition by holding them at relatively high temperatures. The internal changes occurring during reversal can be compared with those occurring during induction.

Another advantageous feature of the sugar beet is that it builds up a large reserve of stored food that enables it to undergo long periods of storage in darkness. During such storage the internal changes related only to temperature can be studied.

Selection of suitable tissue from the sugar beet, for reliable measurements of the internal changes to be considered, presented some difficulty. Although the growing points of buds or shoots are probably the places where the primary changes in the shift from vegetative to reproductive development occur, the parts sensitive to photoperiodic stimuli are different from those sensitive to thermal-induction stimuli. KNOTT (14) and others have shown with various plants that sensitivity to photoperiod is localized in the leaves. The writer has found this to be true in annual beets. CURTIS and CHANG (8) and CHROBOCZEK (5), working with celery and beets, respectively, have shown that the crown is the region sensitive to the thermal stimulus. It probably can be safely assumed that the substances or stimuli arising in these different parts and at some distance from the growing points are translocated to the growing points and there bring about the changes. An obstacle to the study of bud tissue is the fact that extensive differentiation within a very limited region makes it difficult to obtain comparable samples of tissue for study. There

is in the sugar beet, however, a considerable volume of pith tissue just beneath the apical bud of the crown. This tissue is fairly uniform and because of its proximity to the apical bud might be expected to reflect the biochemical changes occurring in the region of the growing point. The measurements of oxidation-reduction potential and catalase activity were made in this pith tissue just beneath the apical bud.

Material and methods

THERMAL INDUCTION AND REVERSAL IN BIENNIAL BEETS.—The sugar-beet varieties S.L. 234 and S.L. 456 used in the thermal induction and reversal study are both moderately nonbolting varieties. That is, they require prolonged exposure to cool temperature for adequate thermal induction. Variety S.L. 456 is approximately equal in bolting tendency to S.L. 234 (described in a previous report [19]).

The most critical studies on bolting response in relation to biochemical changes were made during the reversal phase of thermal induction. Values of environmental factors, including light duration and intensity and greenhouse temperatures, can be kept more nearly constant during the short time required for reversal than during the much longer time required for induction.

Beets which had overwintered in the field were removed and the crowns carefully trimmed of petioles without injuring the crown buds. The tap roots were cut off at a point where they were about $\frac{1}{4}$ inch in diameter and were washed and blotted with a towel before storage for reversal or for immediate testing. Any new growth of tops during storage at warm temperature was trimmed off before sampling. All lots were allowed to reach thermal equilibrium similar to that of the respiration chambers for 16 hours before being tested.

PHOTOPERIODIC INDUCTION IN ANNUAL BEETS.—The beet used in this study was an F_1 hybrid between a biennial sugar beet and an annual beet (1). This annual was chosen because it had excellent vigor and some curly-top resistance. It usually requires about 3 weeks from the time beets of this hybrid and other annual varieties are placed under continuous illumination for seedstalks to start to develop.

The seed was planted in the field about August 1, after the days were sufficiently short to insure that only vegetative growth would occur during the autumn months. The plants were overwintered in the field and removed from the soil about February 28. Some freezing damage was evident in some of the beets when they were removed to the greenhouse. For this reason they were replanted directly to the warm, short-photoperiodic environment without warm storage to reverse thermal induction. Many of the plants failed to grow owing to frost injury; others developed very elongated crowns or short seedstalks before they reverted to vegetative growth. For this reason it was impossible to make the study as complete as planned, and the elongation of the crowns probably resulted in additional sampling error in some of the measurements of catalase activity and oxidation-reduction potential. The beets were placed under continuous illumination on April 10, 1947, immediately after the initial samples were taken for study.

RESPIRATION.—Determinations of respiration rate were made on approximately uniform weights and equal numbers of beets placed in large, dark glass bottles with carefully calibrated volumes. After a 24-hour period at constant temperature, the gas in the respiration

chamber was analyzed by means of an Orsatt apparatus kept at the same temperature as the chamber. Values of O_2 used and CO_2 evolved were both determined. It is believed that the values of O_2 used are the more reliable owing to the lower solubility of oxygen in the beet tissues. Since most respiration values are based on CO_2, however, the O_2-used values are also calculated on the basis of the molecular weight of CO_2. Other studies have shown that increased CO_2 and lowered O_2 content of the storage atmosphere reduces the respiration rate of sugar beets. The results reported, therefore, tend to show smaller differences between treatments, owing to the method of determination, rather than the larger differences that would have been observed if the measurements had been made in a storage atmosphere of constant composition.

CATALASE.—Catalase activity varies widely in the different crown tissues of beets. The crowns were, therefore, cut off slightly below the apical bud until an area of pith tissue about $\frac{1}{4}$ inch in diameter was exposed. A central cylinder of pith tissue about $\frac{3}{8}$ inch in diameter was then removed by means of a cork borer, the nonpith upper rim of this cylinder was trimmed off, and a $\frac{1}{2}$-gm. sample was cut from the upper end. A similar $\frac{1}{2}$-gm. sample was removed from another beet, and the two were ground in a mortar with a little coarse quartz sand and 1 gm. of powdered $CaCO_3$. The finely ground mixture was made up to 25 ml. with distilled water.

Catalase determinations were made in duplicate within 2 hours after the tissues were ground. Five ml. of 4% H_2O_2 containing a small amount of $CaCO_3$ were placed in one arm of a catalase tube. From the freshly shaken tissue sample $2\frac{1}{2}$ ml. were added to the opposite arm. The tube was fitted tightly to a shaking device, and the water level in the measuring burette was adjusted. After the apparatus was tested for leaks and constant temperature—indicated by no contraction or expansion of the air in the apparatus when under reduced pressure—the mechanical shaking device was started. Oxygen evolution was measured by water displacement at 1-, 3-, and 5-minute intervals. A shaking rate of about sixty-five oscillations per minute was used, since this was the most rapid rate that allowed emptying of each arm of the catalase tubes during a shaking cycle. The reaction rate did not follow that of a unimolecular reaction, probably owing to partial inactivation of the catalase by the H_2O_2. Duplicate determinations on one sample usually agreed within 0.2 ml. of O_2. Duplicate samples from different beets of a given treatment varied much more.

OXIDATION-REDUCTION POTENTIAL.—Several methods were used in a preliminary way early in the study in an attempt to find consistent differences in oxidation-reduction potential between thermally induced and reversed beets. The most consistent differences were observed when the direct measurements were made by the method outlined below. Values obtained by methods other than the one described are omitted from the data.

Progressive transverse slices of the crown were removed, beginning at the apical bud, until the pith tissue was barely exposed. A cylinder of tissue about $\frac{3}{4}$ inch in diameter, containing the pith in its center, was then cut out with a cork borer, and its upper portion, to be used for testing, was cut off slightly more than $\frac{1}{2}$ inch below the top. About $\frac{1}{16}$ inch was then cut off to expose a new top surface. The cylinder was placed, top side up, in

the 5-ml. cup of a Beckman vacuum tube potentiometer, and the cup was raised to insert the platinum electrode firmly and completely into the center of the pith tissue. The cup was then filled with a 15% sucrose solution containing 0.01 *M* KCl. This excluded air and flooded the exposed tissue with a solution approximately isotonic in sugar content with the tissue fluid. The KCl made electrical contact with the saturated calomel half-cell mounted slightly above and to the side of the platinum electrode. The whole operation required only a few seconds from the time the fresh surface was exposed and, therefore, allowed little opportunity for any possible atmospheric oxidation to occur. The E.M.F. value of the cell so formed was quickly balanced with a minimum of current flow and the value recorded. These values are not corrected for the potential of the saturated calomel half-cell but are merely the direct potentiometer readings. The value of any particular concentration gradient potential was not known. The data, therefore, are not absolute values but are merely indicative of relative values. Measurements of approximately ten beets were made at each sampling.

The ordinary method of cleaning the electrodes in fuming $H_2SO_4 + K_2Cr_2O_7$ solution and washing in distilled water led to erratic results for the first measurement following such cleaning. Consequently, the following routine for cleaning platinum electrodes was carefully employed after an initial washing in distilled water: hot distilled water, fuming $H_2SO_4 + K_2Cr_2O_7$, hot distilled water, 0.01 N NaOH, 0.01 N HCl, hot distilled water, then, finally, distilled water at room temperature. The electrodes were kept in distilled water until used. If an electrode was rinsed well in distilled water between successive readings, there was little residual effect of its previous use.

FURTHER OBSERVATIONS ON OXIDATION-REDUCTION POTENTIAL MEASUREMENTS AND ELECTRODES.—Some rather large differences were observed between the potential of the pith tissue and that of the storage tissue below. In induced beets the observed potential was usually more than 100 mv. lower in the pith tissue immediately below the growing point than it was in the region below the pith, while in reversed beets the differences in potential at these same points were not so great. The oxidation-reduction potential was only slightly lower immediately below the pith tissue than it was at positions farther down in the storage tissue in both induced and reversed beets. These observations indicate that the storage tissue of the beet is probably not involved to any great extent with regard to changes in oxidation-reduction potential.

During the course of the earlier work in 1945–46 it appeared that any alteration of the platinum electrode resulted in great differences in measured potentials. When the first electrode was broken and a new one made of heavier platinum wire, the differences in readings were attributed to possible differences in impurities of the platinum. When, however, the exposed platinum wire was shortened, further differences in readings were observed. Electrodes for the 1947 studies were, therefore, made from the same piece of platinum wire and were carefully matched for length of exposed platinum. The average values obtained with two of these electrodes were practically identical during a series of measurements. For example, in the series of measurements just below the growing point (table 3) the over-all average value with electrode no. 1 was 119.2 mv.; with electrode no. 2,

120.5 mv. The average value in storage tissue about ½ inch below the growing point with electrode no. 1 was 224.9 mv.; with electrode no. 2, 224.7 mv. The over-all average of about one hundred and forty measurements in various positions in the beets with electrode no. 1 was 162.0 mv.; with no. 2, 162.6 mv. It appears possible, therefore, to match electrodes so that a series of measurements can be completed in case one electrode is broken.

Results

THERMAL INDUCTION AND REVERSAL IN BIENNIAL BEETS.—Thermally induced beets of variety S.L. 234 were taken from an overwintering plot at Salt Lake City on March 28, 1944 (test I). They were segregated into six lots. One lot was stored at approximately 0° C., while the others were stored at approximately 24° C. At intervals of about 5 days, one lot of about forty beets was taken from the warm chamber. The respiration rate based on fourteen beets, selected to weigh 500 gm., was determined. Twelve beets of the same lot were replanted in a warm greenhouse room under continuous illumination for observation of bolting response. The remainder of the lot was placed in the 0° C. chamber and held for catalase determination after the conclusion of the warm storage period. The same procedure was followed with other lots after different periods of warm storage.

A similar test with overwintered beets was started on May 2, 1944 (test II). In this test catalase activity was determined on the same days that the samples were removed from the warm storage chamber.

Similar results were obtained in both experiments (table 1). Thermal induction was reversed by the high-temperature storage as indicated by a rapid decline in rate and percentage of bolting. After 21 days of warm storage in the first test and 15 days in the second test, all plants remained vegetative after replanting. Typical bolting responses following replanting in a warm greenhouse room under continuous illumination are shown in figure 1. The respiration rate and catalase activity progressively declined as reversal of thermal induction occurred.

In another test started in July, 1945, respiration rate, catalase activity, and oxidation-reduction potential were meas-

TABLE 1

CORRELATION OF REVERSAL OF THERMAL INDUCTION WITH RESPIRATION RATE AND CATALASE ACTIVITY. VARIETY S.L. 234

Days stored at 24° C.	Respiration rate (CO_2/kg. of beets/hour) based on		Temperature (° C.)	Catalase activity (oxygen evolved in 5 minutes)* (ml.)
	O_2 used (mgm.)	CO_2 prod. (mgm.)		
	Test I, started March 29, 1944			
0	85.3	84.3	24.1	.....
5	68.6	64.4	23.1	4.7
10	64.9	59.9	23.2	4.5
15	51.9	46.1	23.6	3.8
21	46.6	42.4	23.8	3.2
Control stored at 0° C.	75.0	73.0	23.0	7.9
	Test II, started May 2, 1944			
0	73.5	70.6	23.4	6.9
3	67.2	62.8	23.0	6.1
10	61.4	58.0	24.2	4.9
15	58.4	53.0	24.4	4.4
Control stored at 0° C.	72.6	69.6	23.2	7.1

* Difference required for significance at 5% level: test I = 0.75; test II = 1.00.

ured in relation both to the thermal induction of beets overwintered in the field and to the reversal of thermal induction resulting from warm storage. The study began on July 13, as soon as spring-planted beets attained sufficient size for some of the physiological measurements. A supply of approximately uniform-sized beets of variety S.L. 456 was obtained by close spacing of beets in one row of a field plot grown for sugar production. After October, beets from an early August planting were used. These beets were too large and widely spaced to insure survival during cold weather, so they were covered with soil about November 17 to protect them from freezing injury. The beets were uncovered and sampled thereafter as needed until March 26, when the remainder were taken from the soil and stored at warm temperatures (average 23.5° C.) during the reversal phase of the study.

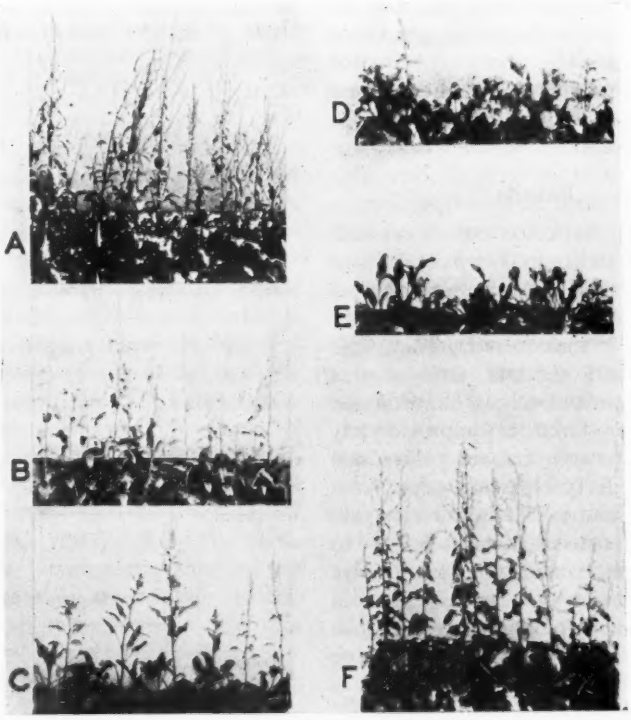

FIG. 1.—Bolting response of beets corresponding to treatments in first test of table 1. *A*, control (no storage); *B*, reversed 5 days; *C*, reversed 10 days; *D*, reversed 15 days; *E*, reversed 21 days; *F*, control stored at 0° C. Photographed 44–45 days after replanting.

The data in table 2 and figure 2 indicate a high degree of correlation between all factors studied, in both the positive and the reversal phases of thermal induction.

Attention should be called to a difficulty that arose in the measurement of

oxidation-reduction potential during the test. The platinum electrode that was used until February 5 was broken and a new electrode was made from heavier platinum wire. The E.M.F. values obtained with the new electrode were higher than those with the original electrode, but the change in relative values is the important consideration. In order to show the relative changes in figure 2, an arbitrary point was chosen for the reading for March 27. Other points were established by calculating the differences between the readings and plotting them on the scale used.

TABLE 2

CORRELATION OF THERMAL INDUCTION AND REVERSAL OF THERMAL INDUCTION WITH RESPIRATION RATE, CATALASE ACTIVITY, AND OXIDATION-REDUCTION POTENTIAL. BEETS SAMPLED AT DATES INDICATED. REVERSAL PHASE OF STUDY STARTED MARCH 27, 1946. VARIETY S.L. 456

Sampling date, 1945–46	Days stored at 23°.5 C.	Respiration rate (CO_2/kg. of beets/hour) based on		Temperature (° C.)	Catalase activity (oxygen evolved in 5 minutes) (ml.)	Oxidation-reduction potential (direct potentiometer reading vs. saturated calomel half-cell) (mv.)
		O_2 used (mgm.)	CO_2 prod. (mgm.)			
July 13	.....	67.7	62.9	25.3	2.9	103.2*
Aug. 7	.....	56.9	52.6	25.6	3.6	92.6
28	.....	45.1	43.8	25.3	2.4	138.0
Sept. 19	.....	52.3	50.7	25.3	3.6	124.0
Oct. 9	.....	57.5	56.0	25.2	3.6	111.3
Nov. 16	.....	90.6	86.4	25.4	5.0	42.5
Dec. 18	.....	105.6	100.8	25.5	5.8	9.5
Jan. 10	.....	108.2	104.5	25.5	6.5	− 23.2
Feb. 5	.....	104.6	102.3	25.5	5.6	12.3
March 27	0	98.7	97.3	25.4	5.6	170.1†
April 1	5	86.4	81.8	25.4	3.1	196.3
4	8	72.4	67.5	25.4	2.4	224.8
8	12	60.1	56.4	25.4	2.4	271.1
11	15	49.5	46.2	25.4	2.0	280.3
15	19	50.4	47.5	25.4	.....	.....
18	22	44.2	41.5	25.4	1.8	.....
Difference required for significance at 5% level	.....	7.4	7.2	.....	0.9	32.6

* Platinum electrode No. 1. † Electrode No. 2.

PHOTOPERIODIC INDUCTION IN ANNUAL BEETS.—The data in table 3 show the changes observed in annual beets during photoperiodic induction. There was a rapid rise in respiration rate. The data in regard to catalase activity are not consistent. The change in oxidation-reduction potential during the period of the test was highly significant and in the direction that would be expected from the previous study of thermal induction and reversal and the changes in respiration rate here observed during photoperiodic induction.

Discussion

The fundamental importance of oxidation and reduction reactions, especially in biochemistry, has been recognized since LAVOISIER discovered the analogy of combustion and respiration and the function of oxygen in those processes. The change in concept from one stressing the addition or removal of oxygen from

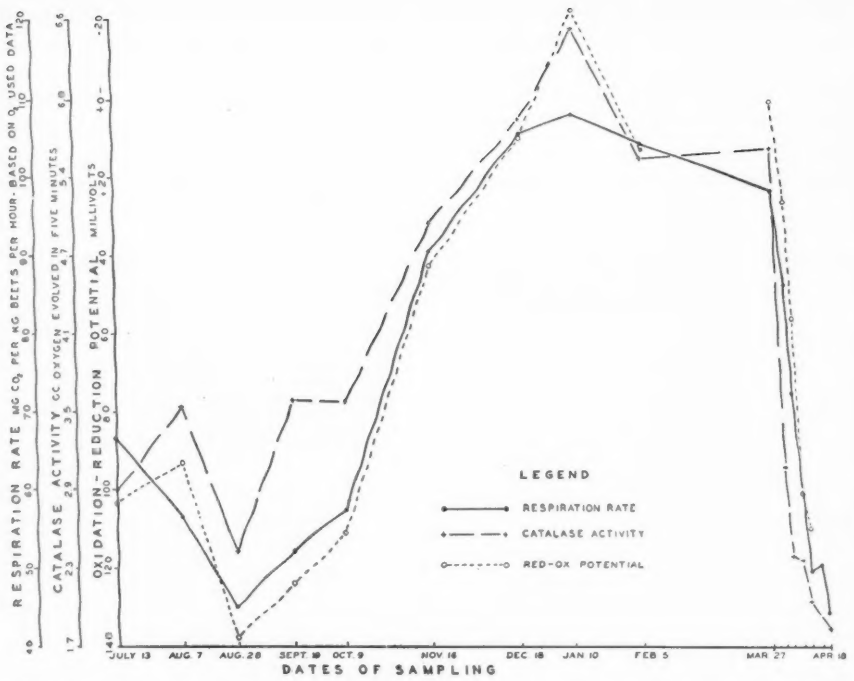

FIG. 2.—Graphical presentation of data in table 2 showing relationship of factors during thermal induction and during reversal (after March 27, 1946).

TABLE 3

CORRELATION OF PHOTOPERIODIC INDUCTION WITH RESPIRATION RATE, CATALASE ACTIVITY, AND OXIDATION-REDUCTION POTENTIAL. ANNUAL VARIETY S.L. 2850

SAMPLING DATE, 1947	DAYS OF CONTINUOUS ILLUMINATION	RESPIRATION RATE (CO_2/KG. OF BEETS/HOUR) BASED ON		CATALASE ACTIVITY (OXYGEN EVOLVED IN 5 MINUTES) (ML.)	OXIDATION-REDUCTION POTENTIAL (DIRECT POTENTIOMETER READING VS. SATURATED CALOMEL HALF-CELL) (MV.)
		O_2 used (mgm.)	CO_2 prod. (mgm.)		
April 10	0	44.2	42.7	2.5	164.3
April 14	4	57.9	57.9	1.7	130.5
April 20	10	88.2	86.9	2.6	95.8
April 24	14	85.9	85.2	2.9	89.9
Difference for significance at 5% level		3.9	5.3	0.7	21.0

compounds to one of addition or removal of electrons has resulted from the acceptance of the modern concept of the atom. In the modern idea of reversible oxidation and reduction (**6**), the reduced state is one relatively rich in electrons in relation to protons, or positively charged nuclei, and, therefore, relatively negative in oxidation-reduction potential. The oxidized state is one relatively low in electrons, capable of accepting others, and, therefore, positive in oxidation-reduction potential. According to this viewpoint, plant respiration is a process in which stored foods that are in a reduced state, and, therefore, rich in electrons, give up atoms or groups of atoms rich in electrons for combination with oxygen, an electron acceptor, and are thereby oxidized. Photosynthesis is a process in which the plant accepts electron-rich groups (CO_2) with the aid of energy in the form of light and forms compounds relatively richer in electrons and, therefore, more highly reduced. In both of these processes plant catalysts (enzymes) capable of initiating the formation of intermediate metastable compounds of intermediate energy levels are necessary to allow the processes to continue at the low energy levels at which they occur. The removal of high activation-energy barriers by plant enzyme processes (**16**) at ordinary temperatures is a mechanism difficult to duplicate by artificial chemical means.

The saturated calomel half-cell (Hg | HgCl, saturated ‖ KCl) has a potential of 245.8 mv. when referred to the hydrogen electrode at 25° C. It is evident that some of the electrodes used in the present study exhibited different degrees of consistency with reference to the standard hydrogen electrode potential. The consistency of a given electrode when cleaned in a consistent manner, however, was fairly uniform, and at least two matched electrodes gave similar values. There seems little doubt that thermal and photoperiodic induction caused an appreciable change in the oxidation-reduction potential of the tissue fluids of certain regions of the plant near the growing point. The change in oxidation-reduction potential was apparently toward the more reduced state as induction toward reproductive development occurred.

The correlation of increased catalase activity with the more reduced state may be interpreted in the following manner: Catalase has the ability to reduce peroxides and possibly other molecular species or radicals capable of accepting electrons and, therefore, oxidizing agents. High catalase activity should keep these compounds at a low equilibrium level and thereby result in a relatively reduced state.

A cool environment greatly reduces the rate of respiration, an oxidation process. From the data it is evident that beets previously exposed to a cool environment respire more rapidly at a given constant temperature than they did before such exposure. It seems reasonable to suspect that other biochemical processes, having different thermal coefficients (Q_{10}) and, therefore, differently affected in rate of reaction by the cool temperature, may reach a state of unbalance as compared with beets grown continuously in a warm environment. However the mechanism of the process occurs, the evidence indicates that the stored foods of thermally induced beets are more rapidly oxidized by the respiration process than are those of non-thermally induced beets; this would seem to indicate a more highly reduced state of the thermally or photoperiodically induced beets.

The physiological factors apparently correlated with induction processes (fig. 2) reached a minimum on August 28. This apparent decline in induction processes occurring from July to August 28 might be correlated with the decreasing photoperiods. After August 28 the predominant influence in increasing induction was cooler temperatures.

It has been observed that sugar beets of the most strongly biennial types—that is, those that require extensive thermal induction—frequently develop seedstalks more readily following favorable thermal environment in the field than following favorable cool-temperature exposure in darkness (19). In this instance it seems probable that thermal induction (reduction) and photosynthetic activity (reduction), even at short photoperiods, complement each other in bringing about a more highly reduced state. Another possibility is that some photosynthetically produced, or photo-activated, substance is translocated from the leaves to the crown and there stimulates the biochemical processes that occur at cool temperatures, thereby resulting in more complete or earlier induction of reproductive development.

Any improvement that can be made to increase the reliability of measurements of oxidation-reduction potential in plant tissues would be highly desirable. The measurements can be made very rapidly by the method outlined, but differences between electrodes and between different beets given the same environmental treatment are usually rather high. Greater consistency might result in the development of a useful diagnostic technique for determining the status of reproductive induction processes.

Summary

1. Respiration, catalase activity, and oxidation-reduction potential were measured in relation to thermal induction of reproductive development (exposure to cool temperatures) and reversal (exposure to warm temperatures) in biennial sugar beets. Similar measurements were made in relation to photoperiodic induction (exposure to continuous illumination) in annual beets.

2. Respiration rate and catalase activity increased and the oxidation-reduction potential decreased as thermal induction progressed. Reversal of thermal induction was correlated with corresponding changes in values of those physiological measurements. Respiration rate increased and oxidation-reduction potential decreased during photoperiodic induction in annual beets. Changes in catalase activity were indefinite in this study.

3. Induction processes are correlated with changes in the oxidation-reduction balance of certain regions of the plant, and these changes seem to agree with present chemical theory. The more reduced state seems to be correlated with an increase in induction toward reproductive development.

U.S. Sugar Plant Field Laboratory
1810 South Main Street
Salt Lake City, Utah

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EFFECTS OF PHOSPHORUS DEFICIENCY ON GROWTH AND METABOLISM OF SUNFLOWER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **605**

SCOTT V. EATON

Introduction

Several investigators have found that phosphorus deficiency interferes with the nitrogen metabolism of plants. ECKERSON (**8**) reported that phosphorus was necessary in the tomato plant for the formation of the nitrate-reducing enzyme, reducase. Therefore, if the element was deficient, protein synthesis was interrupted. Nitrates and carbohydrates accumulated, and the plant assumed the aspects of a nitrogen-deficient plant. KRAYBILL (**18**) found that in addition to carbohydrates and nitrates the water-soluble forms of nitrogen—ammonia, amino acids, and amides—were high in a phosphorus-deficient tomato plant. On the other hand, BREON and GILLAM (**1**) suggested that too much emphasis has been given to the importance of phosphorus as a regulator of nitrogen metabolism. Although they found that nitrates accumulated in tomato plants showing moderate but definite symptoms of phosphorus deficiency, they decided that this resulted from more rapid absorption of nitrates rather than failure of nitrate reduction. RICHARDS and TEMPLEMAN (**31**) stated that the most characteristic effect of phosphorus deficiency in barley leaves was the accumulation of amides rather than nitrates.

The effects of phosphorus deficiency on different plants and on the same plant under various conditions may vary. It was thought that by studying several

plants of different families a better overall picture would be obtained. It was decided to study the same plants that were used in a series of sulfur-deficiency studies—sunflower, soybean, and black mustard. A paper summarizing the effects of sulfur deficiency on these plants and giving the results for black mustard was published in 1942 (**6**). The present paper reports the effects of phosphorus deficiency on the sunflower.

Material and methods

Sunflower plants (*Helianthus annuus* L., var. Mammoth Russian) were grown in pure quartz sand in 2-gallon glazed earthenware crocks each of which had a hole in the bottom for drainage. The hole was covered with glass wool upon which was placed a small piece of glazed crock to prevent loss of sand. The sand was washed thoroughly with distilled water before planting the seed. An excess of seed was planted on May 8, 1945, and 12 days later the plants were thinned to four uniform plants per pot.

TABLE 1

MOLAR CONCENTRATION OF SALTS OF NUTRIENT SOLUTION

Solution	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	KNO_3	KCl
Minus P . .	0.005	.....	0.002	0.005	0.001
Plus P.....	0.005	0.001	0.002	0.005	.....

Enough of the nutrient solution, which was essentially solution number 1 of HOAGLAND and ARNON (**16**), was applied to the sand daily so that there was some drainage. Previous work (**7**) had shown that, of several solutions tested, it was best for the growth of sunflower. Table 1 gives the concentration of the major salts. Manganese chloride and boric acid were also present at a concentration of 0.5 p.p.m. of manganese and boron. A solution of iron citrate was applied to the sand once a week and solutions of zinc chloride and copper chloride every 2 weeks at concentrations of 1.00, 0.05, and 0.02 p.p.m., respectively, of iron, zinc, and copper.

SAMPLING AND EXTRACTION

The plants were sampled on May 29, June 6, and June 20. The number of minus-phosphorus and plus-phosphorus plants were, for the first harvest, 116 and 52, respectively; for the second, 84 and 52; and, for the third, 84 and 51. The first harvest was made rather early before phosphorus-deficiency symptoms had become extreme. At this time the minus-phosphorus plants were decidedly smaller and darker green than the plants grown in a complete nutrient solution, but there was no chlorosis except in a few of the cotyledons. In their work on potassium deficiency PHILLIPS *et al.* (**28**) pointed out that plants in an advanced stage of deficiency are quite stunted and abnormal. It is difficult to compare them with normal plants, and misleading results may be obtained.

The stems only were analyzed. They are usually more constant in their chemical constitution than the leaves and probably give a better picture of the effects of a deficiency on the plant. The stems were divided into three equal lengths. Each length was cut into small pieces, and weighed samples were transferred to Erlenmeyer flasks containing a small amount of calcium carbonate. Enough hot 95% alcohol was added to make the final concentration 80%, and the contents were boiled on a steam bath for 10 minutes. Any alcohol lost during the heating was replaced.

The alcohol used to preserve the samples was filtered, and the residue was covered with 70% alcohol. After heating to boiling and cooling to room tempera-

ture the extract was filtered. Seven such extractions were made. The extracts were combined, made to volume, and stored in an Erlenmeyer flask.

Methods of analysis

Carbohydrate fractions.—The alcohol was evaporated from a suitable aliquot of the extract on a steam bath, and the aqueous solution was cleared with neutral lead acetate and deleaded with potassium oxalate. In the determination of total sugars including sucrose the sucrose in 10-ml. aliquots was inverted with invertase by the method of Phillips and Smith (**29**). The difference between the reducing power before (reducing sugars) and after (total sugars) the inversion was regarded as a measure of sucrose. All sugar determinations were made by the method of Phillips (**27**). Ten-ml. aliquots were heated on a steam bath at 100° C. in large, loosely stoppered test tubes. Phillips' sucrose equation was used in calculating both total and reducing sugars. The equation was checked with Bureau of Standards' sucrose, and results quite close to those given by Phillips were obtained.

Starch was determined by the salivary digestion method. The residue of the alcoholic extraction was transferred to a 400-ml. beaker and dried to constant weight in a vacuum oven. It was ground first to 20 mesh and then to 80 mesh in a semimicro Wiley mill and again dried to constant weight. A suitable aliquot was transferred to an Erlenmeyer flask, water was added, and the starch was gelatinized by heating on a steam bath for an hour. The extract was cooled to 37° C., 5 ml. of filtered, fresh saliva diluted 1:1, and a few drops of toluene were added, and the mixture was allowed to stand overnight at room temperature. It was heated to boiling, filtered through a porous filter paper, and the residue washed with hot water. Clearing and deleading were carried out as described above. An aliquot was heated in an autoclave at 15 pounds pressure for an hour with 2½% HCl to hydrolyze the maltose. The solution was neutralized with NaOH, and the sugar was determined by the method of Phillips (**27**). The starch was calculated as glucose, using his glucose equation, which was checked with the Bureau of Standards' glucose.

Nitrogen fractions.—Total nitrogen including nitrates was determined in the alcoholic extract by the method of Pucher *et al.* (**30**), adapted to the semimicro Hengar Kjeldahl method. The digestion and distillation were carried out according to the directions given by the Hengar Company. The Hengar method was also used in determining the nitrogen content of the alcoholic residue.

The nitrogenous fractions—ammonia N, amide N, amino N, and nitrate N—were determined in the alcoholic extracts of the second harvest only. Methods previously described (**5**) were used except that ammonia was determined not by vacuum distillation as in the previous work but by aeration in a Van Slyke–Cullen urea apparatus.

Phosphorus fractions.—Total phosphorus in the alcoholic extract and residue was determined essentially by the method of Cotton (**3**). When the digestion was complete, the solution was made up to 100 ml. in a volumetric flask. An aliquot was transferred to a 50-ml. volumetric flask, the reagents were added, and the solution was made up to the mark. After 30 minutes the reading was made on a Klett colorimeter. A lower acidity than that recommended by Cotton was used, for otherwise the blue color developed slowly and was not very constant after standing 30 minutes.

Results

APPEARANCE AND SIZE OF PLANTS.—An early symptom of phosphorus deficiency was the darker green color of the leaves as compared with plants grown in a complete nutrient solution. This was evident 2 weeks after planting the seed, although at this time there was not much difference in plant size in the two sets. Later the lower leaves of minus-phosphorus plants became chlorotic. The chlorosis generally appeared first at the tips and edges and then extended inward. It was later followed by necrosis of the tissue. The chlorosis and necrosis gradually extended up the stem. Another type of injury to the leaves was that the tissue of certain green areas collapsed, later turning black or dark-brown, drying out and becoming brittle.

Minus-phosphorus plants were smaller than plus-phosphorus ones, with smaller leaves and thinner, harder stems. At later stages of deficiency the difference in plant size in the two sets was very great (table 2). Figures 1, 2, and 3 show the size and general appearance of the minus-phosphorus and plus-phosphorus plants of each harvest. Roots of phosphorus-deficient plants were much darker brown, and there was poorer tap-root development than in the case of plants grown in a complete nutrient solution.

The sunflower plant is apparently able to grow to maturity in a minus-phosphorus nutrient solution. Some plants were grown 64 days in such a solution. At the end of this time only the leaves quite near the top were normal. The leaves of the lower three pairs (or whorls of three) were completely dead, and leaves higher on the stem had chlorotic and necrotic areas. All the plants had developed flower buds. Other plants were grown 49 days without phosphorus. At this time they were suffering from rather extreme phosphorus deficiency. Phosphorus was then supplied. Much new growth developed at the top, and the stem increased in diameter throughout its length.

WEIGHT OF STEMS, PERCENTAGE WATER, PERCENTAGE DRY WEIGHT.—Table 2 gives the fresh weight of the stems and their percentage of water and of dry weight. Weights of leaves and roots were also determined, but, since the weights of these parts differed in a manner similar to the stems, the data are not given. The phosphorus-deficient stems were much smaller than the stems of plants grown in a complete nutrient solution, the difference increasing at the later harvests. The minus-phosphorus stems had a lower water content (a greater percentage dry weight) than the plus-phosphorus ones. In the second and third harvests the water content of the former stems decreased from the top to the bottom of the plant. This was not true of the plus-phosphorus plants.

Phosphorus deficiency affects tops more than roots. This is indicated by a lower top-root ratio of minus-phosphorus plants as compared with those grown in a complete nutrient solution. These ratios were, for the first harvest, 2.177 and 6.427, respectively; for the second, 1.460 and 6.427; and for the third, 1.757 and 6.551. Because of the death of some of the phosphorus-deficient leaves, which were not saved, this difference in the ratios in favor of the plus-phosphorus plants is exaggerated. A more accurate comparison would be obtained if dry weights of tops and roots were used in calculating the ratios.

TOTAL SOLIDS, NITROGEN FRACTIONS.—All the chemical constituents were calculated on three bases: dry weight, fresh weight, and absolute, or weight per 100 plants. To save space, however, only the

TABLE 2

FRESH WEIGHT OF STEMS (GRAMS PER 100 PLANTS), PERCENTAGE WATER, PERCENTAGE DRY WEIGHT

STEM LEVEL	FRESH WEIGHT		PERCENTAGE WATER		PERCENTAGE DRY WEIGHT	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest					
Upper	41	102	92.8	95.1	7.2	4.9
Middle	50	119	93.0	95.6	7.0	4.4
Lower	54	119	93.0	95.4	7.0	4.6
	Second harvest					
Upper	72	453	91.3	94.9	8.7	5.1
Middle	80	517	90.3	95.6	9.7	4.4
Lower	96	608	89.9	95.9	10.1	4.1
	Third harvest					
Upper	84	1703	90.4	93.8	9.6	6.2
Middle	102	2464	88.4	94.2	11.6	5.8
Lower	133	2946	86.7	93.4	13.3	6.6

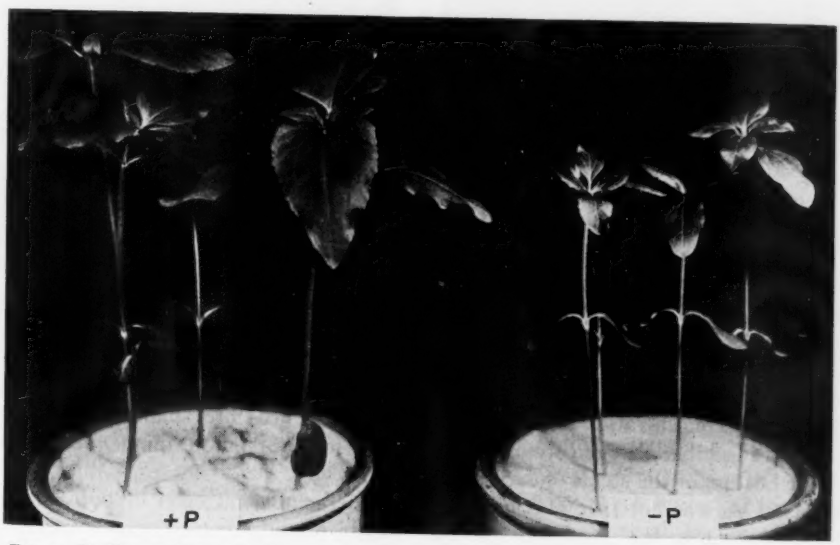

FIG. 1.—Sunflower plants at first harvest, 20 days from planting of seed. (Photograph by P. D. VOTH.)

dry-weight data are given, although some references will be made to the other data. Because of the greater percentage dry weight of the minus-phosphorus stems, their percentage composition was lower in comparison with the plus-phosphorus ones than it is when figured on a fresh-weight basis.

Table 3 gives the data for soluble solids and nitrogen fractions. For total soluble in 70% alcohol in each group of plants. The larger percentages of the nitrogen fractions in the plus-phosphorus stems should not be emphasized, for with

FIG. 2.—Sunflower plants at second harvest, 30 days from planting of seed. (Photograph by P. D. VOTH.)

FIG. 3.—Sunflower plants at third harvest, 42 days from planting of seed. (Photograph by P. D. VOTH.)

ids there was no consistent difference between plus- and minus-phosphorus stems. When figured on a fresh-weight basis, however, they were considerably higher in the latter stems. All the nitrogen fractions were consistently higher in the stems of plants grown in a complete nutrient solution. There was, in general, a decrease in concentration of total nitrogen and insoluble nitrogen from the top to the bottom of the stems of each set of plants. This was not true of soluble nitrogen except in the minus-phosphorus stems of the third harvest. A rather large proportion of the total nitrogen was soluble in 70% alcohol in each group of plants. The larger percentages of the nitrogen fractions in the plus-phosphorus stems should not be emphasized, for with the data figured on a fresh-weight basis the differences are small and in some cases in favor of the minus-phosphorus stems.

The soluble nitrogen of both sets of plants of the second harvest was fractionated into ammonia nitrogen, amide nitrogen, amino nitrogen, and nitrate

nitrogen. Very little ammonia and no measurable amide nitrogen were found in either group. Nitrate nitrogen was higher in the plus-phosphorus stems than in the minus-phosphorus ones when figured on the dry-weight basis, while the percentages of amino nitrogen were greater in the former stems on both bases.

levels except the lower. There was relatively little sucrose in the stems of either set of plants at any time. Reducing sugars made up a large part of the total sugars. Starch was also rather low in the stems of each group. When the data are figured on a fresh-weight basis, the differences in favor of the phosphorus-deficient stems are accentuated. Except for the somewhat higher content of total sugars and reducing sugars in the plus-phosphorus stems at the upper level of the third harvest, the percentages of all the carbohydrates were larger in the minus-phosphorus stems.

TABLE 3

SOLUBLE SOLIDS, NITROGEN FRACTIONS (PERCENTAGES ON DRY-WEIGHT BASIS)

Stem level	70%-alcohol-soluble solids		Total N		70%-alcohol-soluble N		70%-alcohol-insoluble N	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest							
Upper	39.6	40.0	3.27	5.61	2.05	3.79	1.22	1.82
Middle	42.5	43.5	3.09	5.07	2.35	4.04	0.74	1.03
Lower	37.6	39.5	3.06	4.57	2.35	3.60	0.71	0.97
	Second harvest							
Upper	47.0	47.2	2.15	4.26	1.34	2.77	0.81	1.48
Middle	45.4	43.8	1.76	4.17	1.31	3.31	.45	0.86
Lower	40.6	37.6	1.76	3.64	1.34	2.76	0.42	0.88
	Third harvest							
Upper	40.6	45.8	2.02	2.93	1.35	1.88	0.68	1.05
Middle	37.6	39.2	1.44	2.58	1.08	1.96	.36	0.61
Lower	34.3	30.4	1.30	2.29	0.98	1.72	0.32	0.57

CARBOHYDRATE FRACTIONS.—In table 4 are recorded the data for the carbohydrate fractions. Total sugars, reducing sugars, and sucrose were much higher and starch somewhat higher in the minus-phosphorus stems of the first harvest at all levels than in the plus-phosphorus ones. The differences were less in the later harvests and in some cases were reversed. For example, in the third harvest all fractions except sucrose were higher in the latter stems at all

PHOSPHORUS FRACTIONS.—All these fractions were much higher in the plus-phosphorus stems of all harvests than in the minus-phosphorus ones (table 5). In each set there was more of each fraction

TABLE 4

CARBOHYDRATE FRACTIONS (PERCENTAGES ON DRY-WEIGHT BASIS)

Stem level	Total sugars		Reducing sugars		Sucrose		Starch	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest							
Upper	7.74	0.83	5.83	0.82	1.90	0.01	0.86	0.61
Middle	7.78	.41	4.41	.25	3.39	.16	.99	.50
Lower	3.94	0.29	2.77	0.21	1.17	0.08	0.81	0.34
	Second harvest							
Upper	18.97	11.03	16.72	8.67	2.26	2.36	1.04	1.20
Middle	15.98	5.20	12.45	4.50	3.53	0.71	1.02	0.87
Lower	10.50	2.96	7.96	2.34	2.55	0.62	1.00	0.80
	Third harvest							
Upper	11.91	21.53	10.37	20.05	1.54	1.48	0.87	1.04
Middle	13.15	13.60	11.19	12.86	1.96	0.75	.58	0.64
Lower	9.39	6.80	7.84	5.90	1.55	0.90	0.68	0.43

TABLE 5

PHOSPHORUS FRACTIONS (PERCENTAGES ON DRY-WEIGHT BASIS)

Stem level	Total P		70%-alcohol-soluble P		70%-alcohol-insoluble P	
	Minus P	Plus P	Minus P	Plus P	Minus P	Plus P
	First harvest					
Upper	0.19	0.71	0.08	0.19	0.10	0.52
Middle	.12	.53	.06	.17	.06	.36
Lower	0.11	0.57	0.06	0.18	0.05	0.38
	Second harvest					
Upper	0.11	0.63	0.05	0.20	0.06	0.42
Middle	.05	.43	.02	.14	.03	.29
Lower	0.06	0.52	0.02	0.17	0.03	0.35
	Third harvest					
Upper	0.09	0.48	0.05	0.14	0.05	0.34
Middle	.05	.34	.02	.11	.02	.22
Lower	0.04	0.34	0.02	0.10	0.02	0.23

at the upper level than at the other levels, although there was not in all cases a regular decrease from the top to the bottom of the stem. With the data figured on a fresh-weight basis the situation was similar, but the differences in favor of the plus-phosphorus stems were smaller. More of the total phosphorus was present in a soluble form in the phosphorus-deficient stems than in the stems of plants grown in a complete nutrient solution. In general, all the fractions were lower in both sets at the later harvests.

Discussion

Deficiency symptoms

Symptoms of phosphorus deficiency in the sunflower may be divided into two phases. In the first phase the leaves are small and green, and the stems are short, thin, and hard in comparison with plants grown in a complete nutrient solution. The difference in greenness is apparent even before there is much stunting.

A number of investigators have observed the intensified green color of leaves of phosphorus-deficient plants (**18, 19, 21, 31**). McMurtrey (**21**) stated that the leaves of tobacco plants grown in soils deficient in phosphorus are very dark green in color, narrow in proportion to length, and form an acute angle with the stem. It is emphasized that the growth effects of phosphorus deficiency are less characteristic than those owing to the shortage of any other element. Apparently in most crops under field conditions the stunting of the plant and the abnormally green color of the leaves are the main effects observed (**15**). Phosphorus is usually not so deficient in the soil that there develop the extreme symptoms of the second phase described below. Accompanying the darker-green color of phosphorus-deficient leaves there is often a development of anthocyanin. This was not noticed in the sunflower but has been reported by other investigators (**8, 18, 19, 20, 31**).

Conflicting results have been reported for the tomato. Kraybill (**18**) noticed the deeper-green color of phosphorus-deficient plants. On the other hand, Eckerson (**8**) found that 3 weeks after the beginning of minus-phosphorus treatment the plants resembled minus-nitrogen plants but that later the leaves became a deeper green.

In the second phase of phosphorus deficiency in the sunflower the lower leaves become chlorotic, and the chlorosis is later followed by necrosis of the tissue. Necrotic areas may also develop in green parts of the leaves. Chlorosis as a symptom of phosphorus deficiency has not been emphasized by many workers, although several investigators wrote of the dying of the leaves owing to a shortage of phosphorus. Lineberry and Burkhart (**19**) found that in advanced stages of phosphorus deficiency the older leaves of strawberry plants were brown and dry. Richards and Templeman (**31**) recorded that the older leaves of barley plants grown with reduced amounts of phosphorus died early. Probably the death of the strawberry and barley leaves was preceded by a chlorotic stage. Haas (**14**) noted both a chlorosis and a burning of citrus leaves owing to a shortage of phosphorus.

It was stated above that the stems of minus-phosphorus sunflower plants were hard. No anatomical studies were made. Eckerson (**8**) studied the anatomy of phosphorus-deficient tomato stems and found that in comparison with the stems of plants grown in a complete nutrient solution the cell walls of the collenchyma, xylem parenchyma, and pith were thicker. Also the amount of collenchyma was greater. Cambial activity ceased,

and there was less cell division in stem and root tips.

EXPLANATION OF PHOSPHORUS-DEFICIENCY SYMPTOMS.—It is rather difficult to explain the increased greenness of phosphorus-deficient leaves. It may be that there is a greater reduction in size of cells of the leaves than there is in the chlorophyll content per cell. If this is true, the leaves would be greener. ECKERSON (8) found that phosphorus deficiency resulted in a reduction in size of pith and cortical cells of tomato stems.

The greater anthocyanin development of some minus-phosphorus plants in comparison with plants grown in a complete nutrient solution may be regarded as a secondary phenomenon resulting from the accumulation of carbohydrates and the presence of the genic constitution for the development of the pigment. Several investigators have found a correlation of anthocyanin development and the accumulation of carbohydrates in phosphorus-deficient plants (8, 18, 20). Although minus-phosphorus sunflower plants are high in carbohydrates, anthocyanin does not develop, apparently because of the lack of the necessary gene. Anthocyanin development is not specific for phosphorus deficiency. Carbohydrates accumulate in plants deficient, respectively, in sulfur (6, 23), nitrogen (24), and potassium (25), and there is anthocyanin development if the plant has the potentiality for its development.

The high carbohydrate content of minus-phosphorus sunflower stems seems also to account for their hardness. ECKERSON (8) found that the thicker cell walls of various tissues of phosphorus-deficient tomato stems were correlated with a higher carbohydrate content. Again, this phenomenon is not specific for phosphorus deficiency. In the case of any one of the deficiencies mentioned above the accumulation of carbohydrates may result, at least at certain stages of development of the plants, in a thickening of the cell walls of various tissues.

The second phase of phosphorus deficiency in the sunflower, characterized by chlorosis followed by necrosis of the lower leaves, may result from the breakdown of certain phosphorus-containing constituents of the cells. ECKERSON (8) found that, when phosphorus deficiency became acute in the tomato, there was a breakdown of the compounds: starch, phosphatides, and proteins. Both the nucleoproteins of the nucleus and the lecithoproteins of the cytoplasm contain phosphorus (11). Presumably there are also phosphorus-containing proteins in the protoplasm of the chloroplasts so that as a result of a shortage of phosphorus there is probably a disintegration of the chloroplasts as well as of the nuclei and cytoplasm. Amino acids and amides had not accumulated in the minus-phosphorus stems at the time of the second harvest, but the leaves were not analyzed. The situation in them may have been different from that in the stems. ECKERSON (8, 9) found that phosphorus was necessary for the formation of the nitrate-reducing enzyme, reducase. That the chlorosis of the leaves of phosphorus-deficient sunflower plants is not due to the failure of nitrate reduction is indicated by the lack of accumulation of nitrates in the stems, at least at the time of the second harvest.

TOP-ROOT RATIO

It is often stated that phosphorus fertilizers favor root development, nitrogenous fertilizers leaf development. RUSSELL (32) emphasized the effect of phosphorus on the growth of roots. But both

elements are needed for the growth of roots and leaves. To answer the question of whether nitrogen has a special relationship to leaf development and phosphorus to root development, the top-root ratios should be determined. If additional nitrogen supply results in a greater increase in growth of tops than of roots, the top-root ratio should increase; if phosphorus has the reverse effect, it should decrease.

MILLER (22) stated that nitrogenous fertilizers increase the growth of the tops of plants more than the roots. On the other hand, additional phosphorus generally induces greater growth of roots than of tops, decreasing the top-root ratio. It seems to be generally agreed that an increase in nitrogen supply results in an increase in the top-root ratio. In the present work this was also the effect of phosphorus. The plus-phosphorus plants had a higher top-root ratio than the minus-phosphorus ones. This seems to be in accord with many of the results in the literature. RUSSELL (32) stated that the addition of phosphates to phosphate-starved cereals induces a greater growth of tops than of roots. In soil cultures GOEDEWAGEN (10) obtained similar results with wheat and horse bean. With increasing phosphate supply the growth of both shoots and roots increased but the former to a greater extent than the latter. With a rather large phosphate supply the weight of roots remained constant or was even less, but the weight of shoots was greater, so that the top-root ratio increased. It is concluded that phosphorus affects the top-root ratio in essentially the same way as nitrogen. In solution cultures SOMMER (33) obtained similar results with several kinds of plants.

On the other hand, TURNER (34) found that increased amounts of phosphate in the nutrient solution resulted in a decrease in top-root ratios of barley, wheat, and cotton. It is stated that this decrease could result from either a retardation of top growth or an increase in the root growth. Since in sterile-culture experiments corn root tips grew less and produced fewer secondary roots with a large amount of phosphate in the nutrient solution than with a low amount, it is concluded that there is not a direct effect on the roots but that with a high phosphate supply compounds are formed in the tops in connection with photosynthesis. These are translocated to the roots and there bring about more growth or storage.

In discussing the conflicting results of TURNER both GOEDEWAGEN (10) and SOMMER (33) emphasized the large amount of phosphate in his high-phosphorus solution. By growing oats in a small volume of soil supplied with an excessive amount of phosphate, GOEDEWAGEN demonstrated that under these conditions there was a decrease in the top-root ratio. This may have resulted from a toxic effect of the phosphorus. Maximum growth of plants may be obtained with very low amounts of phosphorus in the nutrient solution (26). At high concentrations of the element, therefore, toxic effects would be expected. Just as in the nontoxic realm, an increase in the amount of phosphorus seems to induce greater growth of tops than of roots, so in the toxic realm the element may be more toxic to the tops than to the roots. In the first case the top-root ratio increases; in the second it decreases. It may be concluded that, except at high concentrations, increasing the amount of phosphorus results in an increase in the top-root ratio. The element thus acts in the same way as nitrogen.

Effects of phosphorus deficiency on chemical constitution

Carbohydrate fractions.—Carbohydrates accumulate in the stems of minus-phosphorus sunflower stems, especially in the early stages (table 4). This is in accord with the work of other investigators (**8, 12, 18, 20**). Eckerson (**8**) interpreted the increase in the carbohydrate content of the tomato plant suffering from phosphorus deficiency to result from an interference with protein synthesis at the nitrate-reduction stage. When the phosphate and the water-soluble organic phosphorus compounds are used up, there is no reducase activity. Both nitrates and carbohydrates accumulate.

On the other hand, Richards and Templeman (**31**) found an accumulation of amino acids and amides, especially amides, in low-phosphorus barley leaves before there was much increase in nitrates. They stated that at this same time there was a large accumulation of reducing sugars according to the work of Gregory and Baptiste. It was suggested that phosphorus deficiency interferes with the synthesis of proteins beyond the amide stage, and, therefore, sugars accumulate.

Neither of the above explanations satisfactorily accounts for the accumulation of carbohydrates in minus-phosphorus sunflower stems in comparison with plus-phosphorus ones. At the time of the second harvest (and presumably at the first harvest) when very decided effects of phosphorus deficiency were evident, neither nitrates nor amides had accumulated in the stems. Apparently neither nitrate reduction nor synthesis of proteins from amides was interrupted. Perhaps the higher concentration of carbohydrates in the stems of phosphorus-deficient plants may be at least partly explained not by any disturbance in the metabolism but by the greater photosynthesis in the leaves of these plants relative to the weight of the stems than in the plus-phosphorus leaves. At the time of the first harvest the leaves of minus-phosphorus plants weighed more than the stems. The opposite was true of plants grown in a complete nutrient solution. Also Gregory and Richards (**13**) found that photosynthesis in young, phosphorus-deficient barley leaves was slightly supernormal compared with plus-phosphorus leaves. This may be true in the sunflower. With a greater weight of leaves and a higher rate of photosynthesis it is to be expected that the minus-phosphorus stems would be higher in carbohydrates than the plus-phosphorus ones.

There was less difference in carbohydrate content of the stems of the two sets of plants at the later stages; at certain levels at the third harvest it was higher for the plus-phosphorus stems (table 4). This may result partly from a relatively greater decrease in the older plants in the weight of the minus-phosphorus leaves relative to the stems than in the leaves of the plants grown in a complete nutrient solution. Although at the first harvest the former leaves weighed more than the stems and the latter less, at the second and third harvests the leaves weighed less than the corresponding stems in each group of plants. The leaf-stem ratio of each set of plants was about the same at the second harvest but considerably less for the minus-phosphorus plants than the plus-phosphorus ones at the third harvest. In other words, at the latter harvest the phosphorus-deficient leaves weighed less relative to the stems than did the leaves of plants grown in a complete nutrient solution. The death of some of the former leaves accounts for part of their lesser

weight. One would also expect that, as phosphorus deficiency became more extreme, the rate of photosynthesis would decrease, as GREGORY and RICHARDS (13) found in barley leaves. The reduced weight of leaves and the lower rate of photosynthesis probably afford at least a partial explanation for the fact that at later stages of development there is less difference in the carbohydrate content of minus-phosphorus and plus-phosphorus stems.

NITROGEN FRACTIONS.—A number of investigators (8, 17, 18, 20, 31, 35) have found that water-soluble nitrogen compounds accumulate in phosphorus-deficient plants in comparison with plants grown with a complete nutrient solution. KRAYBILL (18) reported that these compounds in the case of minus-phosphorus tomato stems included nitrate nitrogen, ammonia nitrogen, amino nitrogen, and amide nitrogen. ECKERSON (8) explained the accumulation of nitrates by the failure of nitrate reduction. As to the amino acids and amides it was stated that they may have been formed early in phosphorus deficiency when there was still some reducase present but when certain conditions necessary for their synthesis into proteins may have been absent or that they may have resulted from the proteolysis of proteins late in phosphorus starvation. As mentioned above, RICHARDS and TEMPLEMAN (31) found that amino acids and amides accumulated in phosphorus-deficient barley leaves before there was much piling-up of nitrates. The protein content of the leaves was low. It was concluded that phosphorus is necessary for the synthesis of proteins from amino acids and amides. This viewpoint supports the first explanation of Eckerson.

Phosphorus deficiency does not seem to interfere with nitrogen metabolism in the sunflower to the degree that it does in some other plants. Neither nitrate reduction nor the synthesis of proteins from amino acids and amides seems to be interrupted. At the time of the second harvest, although the plants were quite stunted, none of these compounds had accumulated in the stems of phosphorus-deficient plants. Pure salts were used in making up the minus-phosphorus nutrient solution, but phosphorus was not entirely excluded. It is possible that the plant grows as much as the amount of phosphorus available—both in the nutrient solution and in the plant in the form of reutilized phosphorus—will allow but that the nitrogen metabolism is not seriously disturbed. The leaves were not analyzed. The situation in them may have been different from that in the stems. It is also possible that if the stems of the third harvest had been analyzed, a disturbance in the nitrogen metabolism of minus-phosphorus stems would have been found.

PHOSPHORUS FRACTIONS.—Phosphorus content was higher in the upper parts of stems of both plus- and minus-phosphorus plants. Others have obtained similar results (2, 20). This situation is to be expected, since phosphorus is a component of the nucleoproteins and of the lecithoproteins of the cytoplasm. The number of meristematic and other living cells is higher in the upper part of stems than at lower levels.

Under minus-phosphorus treatment the higher phosphorus content of the upper parts of plants results from the translocation of phosphorus compounds to these parts from the lower regions. MACGILLIVRAY (20) found in one experiment that 68% of the phosphorus was removed from the lower leaves of phosphorus-deficient tomato plants and 78% in another. In the present work sunflower

plants were grown from seed with a minus-phosphorus nutrient solution until small flower buds had developed, a period of 64 days. At this time they were suffering from extreme phosphorus deficiency, but the leaves near the tops appeared normal. There must have been transfer of much phosphorus from the lower levels of the plants to the upper.

Not all phosphorus compounds are translocated equally readily from the older parts to the growing regions. DeTurk (4) fractionated the phosphorus compounds of the corn plant and determined the concentration of the fractions during its development. It was concluded that the main translocatable phosphorus compounds are the phosphates and the acid-soluble organic phosphorus compounds. The phospholipids, nucleic acids, and nucleoproteins are components of the living matter of cells and are necessary for cell division and the functioning of cells. The data indicated that they cannot be reutilized with the possible exception of a small amount of the phospholipids.

Eckerson's (8) data seem to support DeTurk's conclusion. Three weeks after the beginning of the no-phosphorus treatment phosphates could be found only in the extreme stem tips of tomato plants and in some of the root tips. At about the fifth week phosphorus starvation was very severe, and the organic phosphorus-containing compounds—starch, phosphatides, and proteins—began to break down. The plant soon died. It would seem that the phosphates were readily translocated but that the phosphorus of the organic compounds could not be reutilized, at least rapidly enough to prevent death. It would be interesting to study more completely the phosphorus compounds of the sunflower plant. As stated above, the plants were still alive after 64 days of the no-phosphorus treatment. Perhaps this plant is able to reutilize the phosphorus of complex organic compounds more readily than can corn or tomato.

Summary

1. The effects of phosphorus deficiency on sunflower plants were studied in sand culture. An early symptom was the deeper green color of the leaves. Later the lower leaves became chlorotic, and the chlorosis was followed by necrosis of the tissue. In certain cases the tissue of green areas of the leaves collapsed, turned black or dark brown, and became dry and brittle. The leaves were small, and the stems were short, thin, and hard. Growth of tops was affected more than that of roots, resulting in a lower top-root ratio of minus-phosphorus than of plus-phosphorus plants. Roots of the former plants were much darker brown than those of the latter.

2. Phosphorus-deficient stems were low in moisture but were high in total sugars, reducing sugars, sucrose, and starch, especially in the younger plants. At later stages of development there was less difference in the two sets of plants, and at certain levels of the third harvest all the carbohydrate fractions except sucrose were higher in the plus-phosphorus stems. There was little starch or sucrose in the stems of either group of plants. Reducing sugars made up a large percentage of the total sugars.

3. Phosphorus deficiency does not seem to interfere with the nitrogen metabolism of sunflower stems. When figured on the dry-weight basis, total nitrogen, soluble nitrogen, and insoluble nitrogen were higher at all levels in the plus-phosphorus stems of each harvest than in the deficient ones. On the fresh-

weight basis the differences were smaller and in certain cases slightly in favor of the minus-phosphorus stems. None of the soluble-nitrogen fractions—nitrate nitrogen, ammonia nitrogen, amino nitrogen, and amide nitrogen—accumulated in the phosphorus-deficient stems, at least at the time of the second harvest. These nitrogen fractions have been reported to accumulate in other plants suffering from phosphorus starvation.

4. Total phosphorus, soluble phosphorus, and insoluble phosphorus were much higher in plus-phosphorus stems than in minus-phosphorus ones. The soluble phosphorus of the latter stems made up a much larger percentage of the total phosphorus than in the case of the former. Phosphorus seems to be readily translocated from the lower parts of phosphorus-deficient plants to the upper regions. The upper leaves of plants which had been grown about 9 weeks with a minus-phosphorus solution and which had developed flower buds appeared normal. Other plants which had been grown 7 weeks without phosphorus grew rapidly when phosphorus was supplied.

Department of Botany
University of Chicago

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RELATION OF TEMPERATURE AND DAYLENGTH TO THE INCEPTION OF TREE GROWTH IN SPRING

R. F. DAUBENMIRE

Introduction

The arrival of spring in cool and cold climates, with its accompanying resumption of plant growth, has long attracted the attention of man. Since the invention of the thermometer many attempts have been made to establish quantitative relations between the beginning of growth or flowering and the vernal progression of environmental factors. For a long time these efforts were directed to the temperature factor alone. The discovery of photoperiodism a few decades ago signaled a fresh attack on the problem, and many species have been found which are more sensitive to daylength than to the rising curve of temperature. Still other research has shown that the rate of resumption of growth is materially affected by the relative adequacy of winter weather to meet the cold requirement for overcoming dormancy of certain species.

It is clear that no one factor can be used to explain all variations in the phenology of all plants. It also seems safe to state that, although certain general principles may apply to many plant species, deductive inference will never permit accurate prediction of the ecologic relations of particular species that have not yet been the objects of special study.

The present paper embraces the results of two distinct studies, each concerning the etiology of the resumption of cambial activity in trees native to the temperate zone. In one study the behavior of the

same trees was compared in successive seasons. In the other the problem was approached by means of simultaneous observations of individuals of the same species growing at different altitudes on the same mountain and hence under different temperature conditions.

Growth was considered to be any permanent increase in radius of a trunk that could be detected with an instrument measuring the thickness of approximately the outer centimeter of living tissue to 0.001 inch.[1] Actually no increases are really permanent, for, although the radius of a tree may regularly show small increases on successive dawns, temporary diurnal shrinkages exceeding these increases tenfold may have taken place during each daylight period. Therefore, "permanent increases" are intended to apply only to dawn measurements, and all measurements were taken as soon after dawn as feasible or were taken only during cloudy weather if dawn readings could not be made. In late summer, after all the annual increment of cells has been laid down, a tree may shrink tremendously owing to drought. These drought-induced shrinkages also are not considered as nullifying spring growth, for layers of new cells may have been formed even though the net diameter later decreases.

[1] REINEKE (13) has published a note in which he suggested, on a purely theoretical basis, a change in the technique of tree measurement described by DAUBENMIRE (2). He recommended that the lowest of the three brass screws be set to one side under either of the upper screws, so that the bottom screw would not be directly in the path of conduction to and from the spot on the trunk which is under study. This suggestion is untenable, for it ignores the well-known fact that a great number of trees have spiral grain. His aim could be achieved, therefore, only after occasioning considerable damage to the living tree to determine the direction of spiral. Furthermore, extensive field work by FOWELLS (5) and by DAUBENMIRE and DETERS (4) have shown that the effect of a wood screw on cambial activity is confined to a zone only a few millimeters wide about the metal. Species of *Acer* show abnormal growth for as much as a centimeter upward and downward from a screw, but this much response is unusual and still leaves a safe margin of unaffected tissue between the screw and the spot under study. Dendrometers maintained for periods up to 5 years have induced no abnormalities in any of two dozen Douglas firs which the writer has studied, this showing that REINEKE's experience with one tree of this species is not generally applicable. In consequence, REINEKE's theoretical objections are considered as lacking significance in actual field research, and the technique employed by the writer continues to be identical with that originally described.

Observations

Successive observations on the same trees

In 1944, 1945, and 1946 studies were made in late winter and spring of trees of seventeen species growing on the campus of the University of Idaho at Moscow. Although the campus is situated in a summer-dry prairie climate, trees from widely different climatic areas have been successfully established in closed stands in the arboretum. Two or three individuals of each species or variety were studied, and all results are based on the average measurements. During March, April, and May, when the various species normally begin their growth, they were measured on alternate days. From records kept by a co-operative station of the United States Weather Bureau, a few hundred meters distant from the arboretum, daily maximal and minimal temperatures were obtained.

As a working hypothesis it was assumed that there is a threshold temperature value below which the cambium is inactive. Therefore, the highest of the minimal temperatures for each interval between measurements is the only minimal temperature of definite significance. If the radius increased, it was assumed that at least the higher of the minima exceeded the nocturnal temperature level required for growth. A similar compari-

son was made between radial increases and the higher maximal temperature recorded for each interval. Here, again, only the higher value for the interval has any significance. In plotting these data (figs. 1, 2), the point representing each time interval was placed in the middle of the interval, as designated on the abcissa. Temperature values, on the ordinate, represent the highest values, maxima and minima being shown on different graphs. All three seasons' data are superimposed on the same figure, and different symbols are used to keep the seasons distinct. Triangles represent records for 1944, squares represent 1945, and circles represent 1946. Solid shading of the symbols indicates the attainment of radial measurements greater than any value recorded earlier.

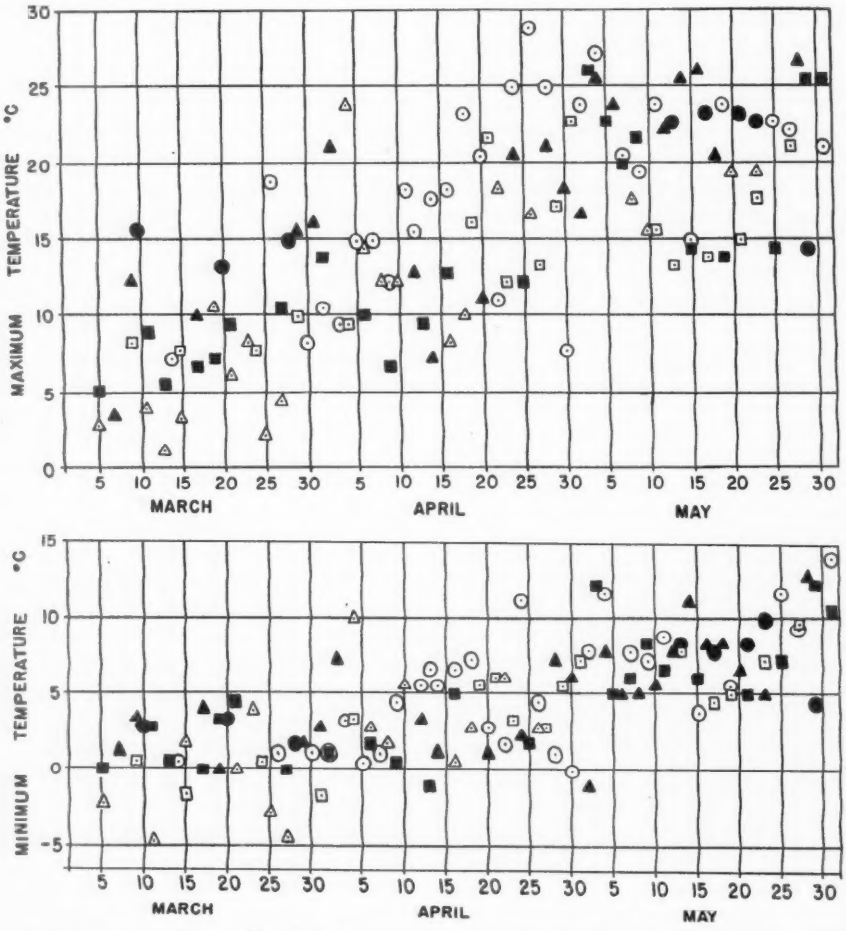

FIG. 1.—Periods of quiescence and of radial enlargement as related to date and air temperature: *Robinia pseudoacacia*. Blackened symbols indicate enlargement. For explanation see text.

Although separate diagrams were prepared for the maximal and minimal temperature data as applied to the growth of each of the seventeen species, only two sets of data are presented (figs. 1, 2). These were selected because they illustrate two extremes that were found in the series of species.

Robinia pseudoacacia (fig. 1) showed no well-defined date of beginning of growth in any year. Small but permanent increases in diameter were recorded sporadically starting early in March. In 1944 growth was continuous after about April 26, but in 1945 and 1946 enlargement was subject to frequent interruption throughout May. It is very evident from figure 1 that short periods of enlargement and quiescence are wholly unrelated to maximal and minimal air tem-

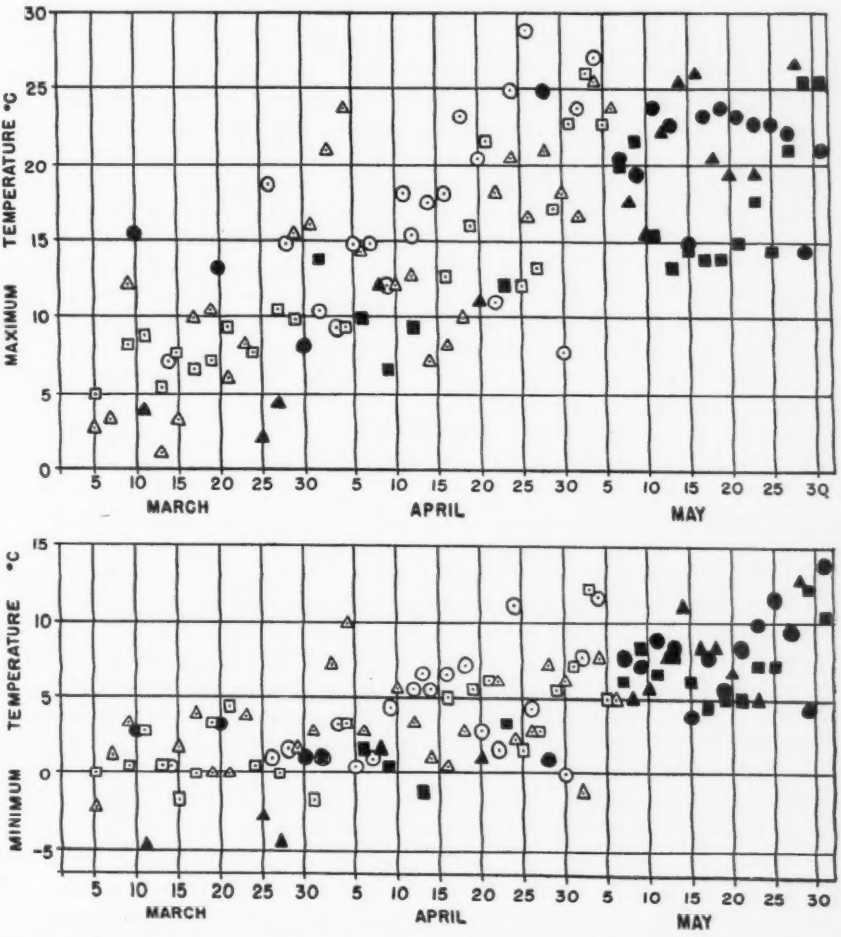

FIG. 2.—Periods of quiescence and of radial enlargement as related to date and air temperature: *Larix occidentalis*.

peratures above −1°.1 C. in March, April, and May.

Larix occidentalis (fig. 2) lies at the opposite end of the series of behaviorisms observed. This species exhibited only sporadic increases before a rather definite date (May 7–8), after which enlargement continued without apparent interruption. After growth began, increases in diameter were observed at times when minimal and maximal temperatures dropped well below levels attained earlier during the dormant period. Thus in *Larix* as in *Robinia*, there is no apparent relationship between growth and maximal and minimal air temperatures during the 3-year period of study. *Larix* differed from *Robinia* principally in exhibiting a rather definite date for the beginning of growth.

NUTTONSON (11) has recently made some calculations in connection with the phenology of field crops which suggest that temperature data might be weighted by daylength to advantage. For a given interval, as between emergence of seedlings and ripening of the crop, he summed the daily mean temperatures in excess of 6°.1 C., then multiplied this sum by the average daylength (sunrise-sunset) during the interval. When the temperature sums, the average daylength, and the calculated products were compared, the last was observed to be "the least variable numerical expression."

NUTTONSON's method was applied to the three seasons' data obtained at the arboretum. The numbers of daylight hours given for the tenth, twentieth, and thirtieth of each month (9) were averaged for the period starting with the first day of the year when the mean daily temperature exceeded 6°.1 C. and ending with the day when pronounced and essentially continuous enlargement began. The result, taken as average daylength, was multiplied by the sum of positive mean daily temperatures in excess of 6°.1 C. for the same period. These three calculations are included in table 1.

It is clear from these data that the technique, which seemed rather promising when applied to crop plants, is of no value for any of the seventeen species of trees that have been studied. In these plants low temperature sums are not compensated by long photoperiods. Possibly there is some fundamental difference between trees and the herbaceous annuals to which NUTTONSON confined his observations. Also it is noteworthy that NUTTONSON did not overestimate his own technique; his multiple was pronounced no more than "the least variable numerical expression." There is indeed considerable variation among the values. For example, in the seventeen values for emergence to heading of Marquis wheat at Moro, Oregon, the lowest value is only 78% of the highest.

The first major conclusion warranted by the data for all seventeen species of trees studied by the writer is that temperature phenomena alone, or temperature data weighted by daylength, cannot be used in explaining the time of their beginning of radial growth in spring. Once rapid growth began, it was maintained during subsequent periods when the weather was actually cooler than warm periods that had occurred just prior to the beginning of growth. The only temperature limitation that is suggested by the data is that *Robinia* made no enlargement at periods when minimum temperatures remained below −1°.1 C. (fig. 1).

A second conclusion may be drawn concerning the character of enlargement in spring. *Robinia* and, to a lesser degree, *Fraxinus* and *Ulmus* appeared to have no well-defined date of beginning of growth.

TABLE 1

DATES WHEN ESSENTIALLY CONTINUOUS ENLARGEMENT BEGAN, TOGETHER WITH DATA FOR CALCULATING EFFECTIVITY OF TEMPERATURE SUMS. SPECIES ARE LISTED IN APPROXIMATE ORDER OF INCREASINGLY BETTER DEFINED TIME OF BEGINNING OF GROWTH

Species	Date of beginning of rapid enlargement	Days of variation	Temp. sum, °F.	Av. hr. daylength	Temp. sum×av. daylength
Robinia pseudoacacia L.	1944 Mar. 7 ?		5.5 ?	9.85 ?	54 ?
	5 Mar. 5 ?	69	23.5 ?	9.57 ?	225 ?
	6 May 13 ?		528.0 ?	11.56 ?	6104 ?
Ulmus americana L.	1944 Apr. 30		233.5	11.32	2643
	5 May 5	31+	250.5	9.56	2395
	6 (after June 1)		805.0*	11.80*	9499*
Picea pungens Engelm.	1944 May 6		302.0	11.32	3419
	5 May 5	20	250.5	11.07	2773
	6 Apr. 16		160.0	10.56	1690
Pinus ponderosa Laws.	1944 Apr. 20		139.0	11.07	1539
	5 May 3	17	212.5	11.07	2352
	6 Apr. 16		160.0	10.56	1690
Acer saccharophorum K. Koch	1944 Apr. 30		233.5	11.32	2643
	5 May 5	13	250.5	11.07	2773
	6 May 13		528.0	11.30	5966
Acer pseudoplatanus L.	1944 Apr. 24		172.5	11.07	1910
	5 May 1	13	167.5	11.07	1854
	6 May 7		418.0	11.07	4627
Pseudotsuga taxifolia var. *glauca* (Mayr.) Sudw.	1944 Apr. 24		172.5	11.07	1910
	5 May 3	13	212.5	11.07	2352
	6 Apr. 20		219.0	10.82	2370
Fraxinus americana L.	1944 Apr. 28		210.5	11.07	2330
	5 Apr. 23	12	104.0	10.82	1125
	6 Apr. 16		160.0	10.56	1690
Fagus grandifolia Ehrh.	1944 Apr. 24		172.5	11.07	1910
	5 Apr. 25	10	113.0	10.82	1223
	6 May 4		385.5	11.07	3925
Juglans nigra L.	1944 May 23		512.5	11.83	6063
	5 May 19	4	395.0	11.30	4464
	6 May 21		650.0	11.56	7514
Pseudotsuga taxifolia (Poir.) Britt.	1944 Apr. 30		233.5	11.32	2643
	5 Apr. 29	4	137.0	10.82	1482
	6 Apr. 26		289.5	10.82	3132
Pinus monticola Dougl.	1944 May 4		269.0	11.32	3045
	5 May 3	4	212.5	11.07	2352
	6 May 7		418.0	11.07	4627
Abies concolor (Gord. & Glend.) Hoopes	1944 Apr. 20		139.0	11.07	1539
	5 Apr. 16	4	51.0	10.56	5386
	6 Apr. 18		596.5	11.30	6740
Picea engelmannii Parry	1944 May 4		269.0	11.32	3045
	5 May 5	3	250.5	11.07	2773
	6 May 7		418.0	11.07	4627
Quercus borealis Michx. f.	1944 Apr. 14		132.5	10.85	1438
	5 Apr. 16	2	51.0	10.56	729
	6 Apr. 14		133.0	10.56	1404
Pinus strobus L.	1944 May 14		406.5	11.56	4699
	5 May 13	1	351.0	11.30	3966
	6 May 13		528.0	11.30	5966
Larix occidentalis Nutt.	1944 May 8		320.0	11.32	3622
	5 May 7	1	275.5	11.07	3050
	6 May 7		418.0	11.07	4627

*Probably more than this value.

Most species, however, exhibited only occasional slight enlargement until a particular season of a few days' duration (table 1) during which they always began rapid and essentially continuous growth. Among these trees, especially *Larix*, *Quercus*, and *Picea engelmannii*, photoperiodic control of growth is strongly indicated.

TABLE 2

LOWEST TEMPERATURES AT WHICH DIAMETRAL INCREASE WAS OBSERVED TO TAKE PLACE AFTER RAPID ENLARGEMENT HAD BEGUN

Species	Lowest min. (° C.)	Lowest max. (° C.)
Deciduous dicotyledons:		
Fraxinus americana	−4.4	2.2
Robinia pseudoacacia	−1.1	3.3
Quercus borealis	−1.1	7.2
Fagus grandifolia	−1.1	12.2
Acer pseudoplatanus	−1.1	13.9
Ulmus americana	−1.1	13.9
Acer saccharophorum	3.9	13.9
Juglans nigra	5.0	13.9
Deciduous conifer:		
Larix occidentalis	3.9	13.3
Evergreen conifers:		
Pinus ponderosa	−1.1	11.1
Abies concolor	−1.1	11.1
Pseudotsuga taxifolia var. glauca	−1.1	11.1
Picea pungens	1.1	11.1
Pseudotsuga taxifolia	1.1	13.3
Pinus strobus	3.9	13.3
Picea engelmannii	3.9	13.3
Pinus monticola	3.9	13.3

Even though the date of beginning of growth for most species may not be predicted closer than a few days, this is actually a well-timed phenomenon, for during spring the day-to-day increase in the photoperiod is very slight, and it seems plausible that cloudy weather at dawn or at sunset might have sufficient effect upon the photoperiod as it reached a critical length to bring about a measurable delay in the reception of the required stimulus. Since observations in the arboretum were made only on alternate days, the apparent difference in dates of beginning of diametral growth in *Larix* may or may not represent a real difference. Consequently, little would be gained in attempting to evaluate relative daylengths in different years, as interpolated from insolation records obtained outside the area of study.

Slight erratic increases in radius in winter, even though they may accumulate to a considerable sum over the entire dormant season, are not to be taken as true growth attributable to the division and immediate enlargement of cambial cells. Some of the enlargement in early winter is undoubtedly due to a rehydration of cells which shrank during the late-summer dry season, for the maximum radius of midsummer is in some cases not attained again until well into winter. Enlargement which carries the radius beyond the summer maximum may be explained as resulting from belated releases of tension set up in the outer bark during the preceding summer when secondary tissue was formed by phellogen and cambium. It is significant in this connection to note that the record for *Fagus* is remarkably free of erratic midwinter increases of small order. These trees had the thinnest rind of dead outer bark of all specimens studied.

In table 2 are listed the lowest temperatures at which diametral increase was observed. In view of the conservative interpretation of the weather data as described earlier, it should be clear that further research may well show lower threshold temperatures for each species. These data, however, are definitely significant in this respect. In evaluating the physiological efficiency of temperature or in ascertaining the length of the temperature-governed growing season, these figures must be used as threshold values until lower values are reported. Dormancy at minimal and maximal temperatures

higher than those indicated for each species must be attributed to factors other than insufficient heat.

It is interesting to note in table 2 that species with low minimal temperature requirements also have low maximal requirements for diametral growth. The consistency of the data suggests that the relationship has some fundamental physiologic significance: that both day and night temperatures are critical.

Simultaneous observations of trees at different altitudes

In 1945 three trees each of three species were studied at three altitudes on Moscow Mountain, Latah County, Idaho. The lowest group of nine trees was situated on the south side of the mountain at an altitude of 1135 meters in the *Abies grandis–Pachystima* association.[2] The second group of nine trees was located on the north side of the mountain at an altitude of 1344 meters in the *Thuja plicata–Pachystima* association. Nine other trees on the same slope were situated at 1463 meters in a stand where *Picea engelmannii* is assuming complete dominance. All trees were large individuals, unshaded from above, but growing in closed stands.

Weekly ascensions of the mountain were made starting March 11. Because of the difficulty of traveling over soft snow, it was impossible to measure all trees at dawn; therefore, the trips in early spring were undertaken only on cloudy days. In such weather in early spring air temperature at the uppermost station commonly did not exceed the necessary minimum of 0° C. upon arrival there, and so the series of comparisons could not be completed. It was felt that none of the essential conditions of insolation and temperature was satisfactory for a critical comparison until the ascent of April 7. Later, when the snow had melted sufficiently that travel by automobile was possible, regular observations early in the morning could be made.

In this study a record was kept of soil temperature at a depth of 20 cm., at least after the snow cover had melted sufficiently so that digging through it was no chore. Growth records in table 3 are expressed as percentages based on the total radial increment measured between April 7 and September 30. Percentages were used in order to minimize the effects of age and competition.

Table 3 shows that snow cover persisted at least 3 weeks later at each successive station progressing up the mountain. Soil temperature, which remained between 0.6 and 1°.0 C. when the ground was covered with snow, also showed progressive change with altitude. If the value of 4°.4 C. is taken arbitrarily as a basis of comparison, the rate of warming of the soil to a given level was delayed about 3 weeks at each station in turn up the mountain.

Growth in *Pseudotsuga taxifolia* var. *glauca* did not commence at successively later dates up the mountain as might be expected if snow cover and temperature were the controlling factors. At the lowest station growth began several weeks *after* the winter snow cover had disappeared, but at the highest station growth began several weeks *before* the snow had melted! The time when growth began was in fact essentially the same at all altitudes, yet this was the time when soil temperatures were most contrasted. The only well-defined altitudinal growth relationship is the slowness of growth at higher altitudes. By June 17 the total seasonal growth was 50% completed at the

[2] Descriptions of forest associations and their classification in this region are in preparation by the writer.

lowest station, 34% completed at the intermediate station, but only 18% completed at the highest. These data substantiate an earlier study of tree growth in relation to altitude on Moscow Mountain (3), although the first study did not begin early enough in spring to record the date when growth began.

The results for *Abies grandis* (Dougl.) Lindl. are essentially identical with those for *Pseudotsuga*. If anything, the lack of harmony between temperature and beginning of cambial activity is even more pronounced.

Diametral measurements of *Larix occidentalis* provided data that are peculiar, but in essence these substantiate principles indicated by both *Pseudotsuga*

TABLE 3

SEASONAL PROGRESSION OF SNOW MELTING AND WARMING OF SOIL IN RELATION TO TREE GROWTH AT DIFFERENT ALTITUDES

Station and species	April		May				June		
	15	29	6	13	20	27	3	10	17
	Percentage of ground covered by snow								
High	100	100	100	100	95	10	0	0	0
Middle	100	100	70	0	0	0	0	0	0
Low	10*	0	0	0	0	0	0	0	0
	Soil temperature in ° C. at 20 cm.								
High	.....	.....	0.8	0.6	0.7	1.0	5.0	4.4	5.0
Middle	.....	0.6	1.3	3.8	4.5	7.3	9.1	7.4	7.7
Low	2.3	4.4	8.0	7.3	6.8	8.2	9.5	9.0	8.9
	Percentage of growth completed								
Pseudotsuga t. var. *glauca* H	2	0	2	10	8	10	12	17	18
Pseudotsuga t. var. *glauca* M	0	4	4	12	12	13	21	28	34
Pseudotsuga t. var. *glauca* L	1	2	5	11	14	21	32	42	50
Abies grandis H	1	0	0	4	5	5	6	12	13
Abies grandis M	0	1	0	4	5	6	12	17	20
Abies grandis L	1	1	1	2	14	23	28	35	39
Larix occidentalis H	0	0	0	0	0	0	0	0	0
Larix occidentalis M	0	0	0	0	4	4	4	17	17
Larix occidentalis L	11	0	0	0	0	0	0	22	22
	Percentage development of *Larix* needles								
High	.....	.....	10	20	30	40	70	80	100
Middle	.....	.....	10	20	30	40	80	80	100
Low	.....	.....	50	80	90	90	100	100	100

* Owing to recent snowfall; ground was bare most of time after March 20.

and *Abies*. The results of this study of three conifers thus indicate that daylength has a stronger control over the inception of growth than does temperature, although the rate of growth in spring, once it begins, is controlled by temperature.

Starting May 6, estimations of the percentage development of *Larix* needles were made, using a binocular field glass. Clearly the foliar development in this species begins before the cambium becomes active, and, although temperature conditions at the bases of the trees differed appreciably between the middle and uppermost station, no differences in rate of foliar development could be detected by the method employed. Either temperature conditions exert no controlling influence over foliar development in *Larix*, or temperature conditions in the canopy are much less correlated with altitude than are temperature conditions at the bases of the trees.

Discussion

The results of the two studies suggest that daylength is more important than temperature in the stimulation of the trees studied to resume cambial[3] growth after winter dormancy. Heat requirements appear to be satisfied earlier in spring than are photoperiod requirements. This lack of direct relationship to temperature is in disagreement with certain other researches which have been concerned with cambial growth in relation to temperature.

MACDOUGAL (**8**) stated that his data indicate a threshold value of 8° C. for cambial growth in *Pinus radiata*. It was only when nocturnal minima remained above this level that radial increase was observed. In the arboretum study only the observations on *Robinia pseudoacacia* suggest the possible existence of such a threshold value.

FOWELLS (**5**) worked on native conifers growing at different altitudes in the Sierra Nevada. He concluded that "the beginning of radial growth was significantly later with each additional 2,000 feet of elevation, with the exception that there was a significant delay between 5,000 feet and 6,000 feet." Although FOWELLS worked with a wider range of altitudes, the climatic gradient in his area, judging from the breadth of the forest zones, is much more gradual than on Moscow Mountain. FOWELLS published no temperature records so that quantitative comparisons in this respect are not possible.

FRIESNER and WALDEN (**6**) kept records for 5 years, March through November, on diametral changes of two trees of *Pinus strobus* growing in Maine. They concluded that "temperature appears to be the most important limiting factor . . . controlling time of initiation of radial enlargement." Certainly their data do not suggest that the photoperiod is critical, for during the 5 years tree A began growth as early as (the week ending) April 15 and as late as May 26: difference, 41 days. Tree B began growth between April 29 and May 15: difference, 16 days.

These workers stated that, with three exceptions out of ten observations, radial enlargement began 1 to 20 days after the mean daily temperature reached 10° C. and remained there essentially continuously. The writer considers these correlations not to be indicative of close temperature control, for in 30% of the observations growth began before the hy-

[3] It should be noted that only *cambial* growth is considered in this discussion. Although growth in the different meristems of the same plant is correlated to a certain extent, periodicities differ, and the relative importance of temperature and the photoperiod may also differ.

pothetical threshold value was attained, and in the remaining instances growth was delayed sometimes as much as 3 weeks.

The results in Maine stand in sharp contrast to the studies of the same species growing in northern Idaho. In 1944 essentially continuous growth began on May 14, and in 1946 the same event was observed on May 13. During the intervening year growth was observed on May 13, 19, 25, and 31, although there was no enlargement on May 15, 17, 21, 23, 27, and 29. Thus the date of beginning of growth was always May 13 or May 14, but in one year growth was intermittent after it began. Temperature relations in these three seasons were quite dissimilar at the time growth began. Maximal values varied from 13°.3 to 25°.6 C., minimal values from 7°.8 to 11°.1 C. Subsequent to the date of beginning of growth, temperatures fell much lower at times, but growth continued unabated; the quiescent periods in late May, 1945, were without relation to temperature. On the other hand, temperatures higher than those prevailing when growth commenced were recorded earlier each season. Results in Idaho, therefore, suggest very close regulation of cambial activity by the photoperiod and warrant a conclusion that, after growth begins, low temperature cannot stop cambial activity except when it falls low enough for frost (4).

OSKAMP (12) studied cambial growth in apple trees in relation to soil temperature at a depth of 23 cm. and, as in the present study, found no correlation.

In view of the divergence of the results that have been obtained when the initiation of cambial activity is studied in relation to temperature and to photoperiod, the conclusion is warranted that neither temperature nor photoperiod alone can be used satisfactorily to explain the date of beginning of activity of this meristem, even in the same species. Possibly some of the difference may be attributable to the inherent genetic differences among the species and ecotypes that have been studied. It is also important that results seem consistent within a given climatic area. Temperature and the photoperiod may exert some influence upon each other, one controlling the beginning of cambial growth only within a given range of variation of the other. Supporting this latter hypothesis are studies of other plant growth responses showing definitely that the two factors are interrelated (1, 7, 10, 14). Herbaceous species commonly respond in a very different manner to the same photoperiod under different temperature conditions, night temperatures being especially important (14).

Summary

1. Two separate studies of the date of beginning of diametral growth in relation to temperature are reported. In one study the behavior of trees of seventeen species of angiosperms and conifers native to the temperate zone was studied in three successive springs. In the other study simultaneous observations were made on individuals of three species of conifers growing at three different altitudes on the same mountain.

2. With one possible exception, the beginning of diametral growth of these trees in spring seems to bear no close relationship to maximal or minimal air temperatures as measured at a standard climatologic station or to soil temperatures as measured at a depth of 20 cm.

3. In most instances growth begins at a rather well-defined period, suggestive of photoperiodism.

4. The only data indicating temperature control of diametral growth were those for *Robinia pseudoacacia*, which was observed to grow only when nocturnal minimal air temperatures did not fall below −1°.1 C.

STATE COLLEGE OF WASHINGTON
PULLMAN, WASHINGTON

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MORPHOLOGY AND CYTOLOGY OF DEVELOPMENT OF THE SEX ORGANS OF PHYTOPHTHORA HIMALAYENSIS DASTUR

BALAJI D. MUNDKUR

Introduction

The phenomenon of sexual reproduction in the Oomycetes has been a subject of great interest ever since the pioneer investigations of DE BARY. A large number of forms, particularly members of the Saprolegniaceae, Pythiaceae, Albuginaceae, and Peronosporaceae, has received much cytological attention, and various schemes of phylogeny have been proposed from time to time based on comparative studies of sexuality (**5, 6**).

It is curious that the genus *Phytophthora* de Bary, in spite of the unique mode of sexual reproduction in a section of its members, has been given scant cytological attention. The only thorough investigation into the cytology of a member of this genus is that of MURPHY (**8**) on *P. erythroseptica* Pethyb. BROWN and EVANS (**1**) described sexuality in *P. cactorum* (Lebert & Cohn) Schroet., but their work was only incidental to a pathological problem, and the account was not given in any considerable detail.

On a basis of the manner in which gametangial copulation is effected, two morphological groups are distinguished among *Phytophthora* species—the "paragynous" and the "amphigynous." The paragynous species, including *P. cacto-*

rum (Lebert & Cohn) Schroet. and *P. syringae* (Kleb.) Kleb., show fertilization involving the apposition of a male gametangium against the larger female gametangium and the passage of the antheridial contents into the latter through a dissolution of the intervening gametangial walls (1). A fertilization tube may or may not occur in this type.

Amphigyny, a condition described almost simultaneously by PETHYBRIDGE (9) and DASTUR (4) is remarkable in that it is a method of gametangial copulation known in only one other genus, the monotypic *Trachysphaera* Tabor & Bunting. Here, an oogonial incept actually pierces the wall of an immature antheridium, penetrates through it, emerges again, and eventually grows into a spherical structure. The antheridium in the mature condition thus completely encircles the stipe of the oogonium as a collar at its base; the term "amphigynous" was first employed by MURPHY (8) to describe such a relation of the gametangia. The amphigynous group includes *P. parasitica* Dastur, *P. infestans* (Mont.) de Bary, *P. erythroseptica* Pethyb., *P. capsici* Leonian, and *P. himalayensis* Dastur.

These morphological conditions, paragyny and amphigyny, led PETHYBRIDGE to erect a new genus, *Nozemia*, to accommodate species of the *cactorum* or paragynous group, while retaining *Phytophthora* to include the amphigynous or *infestans* group. The finding of both paragynous and amphigynous copulations in *P. syringae*, *P. cactorum*, and *P. fagi* (7), as well as their concomitant prevalence in the copulation involving a single oogonium (10), is proof that recognition of the genus *Nozemia* is unwarranted. The present study of copulation in *P. himalayensis* also demonstrates the fundamental similarity of paragyny and amphigyny and provides additional evidence for the untenability of *Nozemia*.

ROSE and LINDEGREN (10), from their study of a *Phytophthora* causing apple and pear rots, questioned the occurrence of amphigynous copulation. They observed an intercalary oogonium which had apparently "grown through" two antheridia, a condition difficult to account for on the basis of existing descriptions of amphigyny. They further stated that "at no time during a rather extensive study of the apple and pear *Phytophthora* have they seen early stages of growth of the oogonium through the antheridium. Antheridia in the amphigynous position have been seen only in the final, fully developed stage and only in the older portions of cultures which were a month or six weeks old." ROSE's and LINDEGREN's skepticism as regards the prevalence of amphigyny, as the term is generally understood, undoubtedly resulted from their failure to observe the earliest stages of gametangial copulation. They seemed to imply that the "amphigynous" relation results from a wrapping of the male gametangium around the female.

COOPER (2) reported that only paragynous antheridia were observed by him in the apple and pear *Phytophthora* (*P. cactorum*), contrary to the findings of ROSE and LINDEGREN, who claimed to have seen both paragynous and amphigynous types in this fungus. Further, COOPER reported that "cultures of *P. erythroseptica* Pethyb., which is described as having only amphigynous antheridia, were obtained from Pethybridge and from the Centraalbureau, Holland, but no amphigynous antheridia were found." Three other Phytophthora species described as amphigynous were found to have only paragynous antheridia. TUCKER (11), however, found that one type al-

ways predominates in cases where the occurrence of both types of antheridia in a single species has been reported and that species with predominantly paragynous antheridia are very easily distinguished from those in which the amphigynous type predominates.

COOPER and PORTER (3) from their descriptions and illustrations conveyed the idea that the amphigynous condition is illusory and that this apparent relation results from a twisting of the antheridium around the oogonial base. According to them, no case was observed where the oogonium actually grew through the antheridium.

There can be no doubt, however, that oogonial incepts do actually grow through the antheridia in the manner in which MURPHY so convincingly demonstrated the phenomenon; my findings in *P. himalayensis* are in complete agreement with those of MURPHY, DASTUR, and PETHYBRIDGE.

Material and methods

The fungus, *Phytophthora himalayensis* Dastur, employed in this investigation was isolated by Professor J. F. DASTUR from diseased potato tubers collected at Kufri, Simla Hills, India. The material was raised on filtered oatmeal agar, Leonian's nutrient agar, and in Leonian's nutrient solution. Growth was best on oatmeal agar. The nutrient solution was unsatisfactory, for, in spite of the fungus being homothallic, it did not form oospores in this solution.

The account of the morphology and cytology of the sex organs is based on a study of serial microtome sections cut at 5 μ and on material stained and mounted whole.

Conventional fixing procedures and staining schedules were used. Bouin's fluid yielded by far the most satisfactory results while Schaudinn's followed closely among several other fixatives tried. For microtome sections oatmeal agar pieces of suitable sizes bearing the fungus were cut out and fixed in order to observe growth and nuclear condition at various stages. Mounting material *in situ*—a method largely used in this investigation—yielded very satisfactory results and at the same time had an advantage over the paraffin-block method in being more rapid. The *in situ* method consists in fixing wefts of mycelium in a fixing fluid like Bouin's, washing out the fixative in the conventional manner, and sticking the mycelium onto the slide directly for staining. Particularly excellent results were obtained with Bouin's fixing fluid which was washed out only partially. This was done by holding the fixed fungus in water in a Petri dish for about an hour. Szombathy's gelatin adhesive was found to be excellent for holding the mycelium onto the slide. For *in situ* mounts two or three drops of Szombathy's adhesive are smeared over a clean slide, and a drop of 2% formalin is rubbed well into the adhesive to harden or "tan" the gelatin. The slide is then placed in a small Petri dish filled with water. The weft of fixed and washed mycelium is transferred to the dish and spread over the gelatin surface of the slide. Gentle teasing facilitates spreading. When the mycelium has spread out sufficiently, the slide is gently lifted out of the Petri dish without disturbing the mycelium and dried partially to allow the fungus to be glued to the slide. It is necessary that the drying should be only partial; the correct stage of drying can be achieved only by experience. If it is dried too much, the material shrinks and is badly distorted. If the time is too short, the material will not adhere to the slide. When the fungus has adhered firmly, it

is ready for staining. Haematoxylin and safranin in oil of cloves as a counterstain yielded by far the most satisfactory results. Flemming's triple combination stain schedule, used with such success by MURPHY, gave consistently poor results with *P. himalayensis*. Both Canada balsam and Euparal were used as mounting media.

The *in situ* mount method, besides being rapid, has the advantage over microtomy in permitting the making of nuclear counts and the observation of fertilization papillae, which is not always possible in the case of sectioned material.

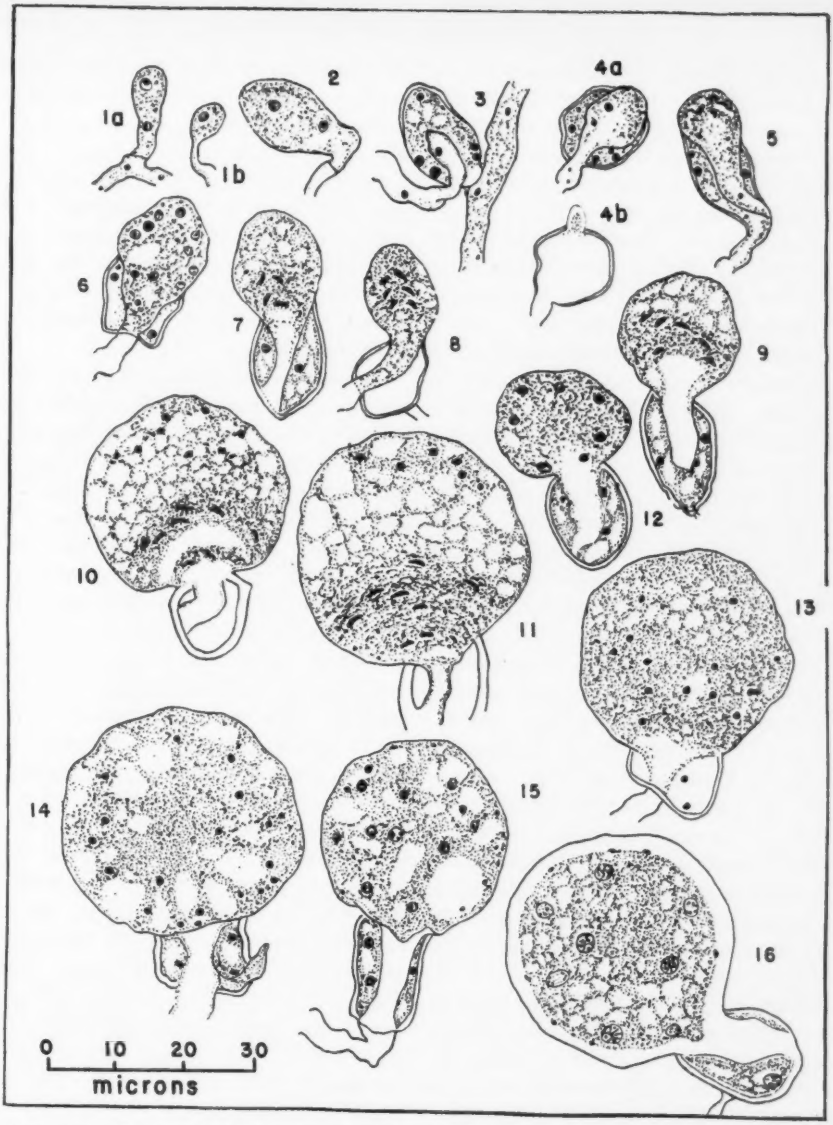
Observations

ONTOGENY OF THE SEX ORGANS

EARLY DEVELOPMENT.—On oatmeal agar the fundaments of the sex organs make their appearance within 4–5 days after making a subculture. The gametangia are formed in large numbers owing to homothallism. The antheridial fundaments appear earlier than the oogonial and arise as clavate swellings both laterally and terminally on the hyphae (fig. 1*A*, *B*). The young antheridium is thin-walled, has granular cytoplasm, and lacks vacuoles. At this stage they have always been observed to possess either a single nucleus or two nuclei, but never more than two. Since the antheridium is not always delimited by a septum from the supporting hypha at such an early stage of growth, it is difficult to ascertain whether two nuclei enter the male fundament from the hypha, or whether a single nucleus divides into two after it has entered the antheridial incept. Young antheridia have been seen in the uninucleate stage both with a septum and without it. Growth of the young antheridium is rapid; it may assume the dimensions of the mature body even before the oogonial incept has penetrated it. In all such cases observed, the antheridium invariably possesses two large spherical nuclei (fig. 2), and nuclear divisions are postponed until after the oogonial incept has entered the antheridium.

MURPHY stated that he had not observed a young antheridium prior to its being pierced by an oogonial incept in *P. erythroseptica*. He, therefore, surmised that in this species the formation of the antheridium was possibly conditioned by contact with an oogonial incept. In *P. himalayensis* several unpierced antheridia have been seen in material mounted *in situ*, but practically none in sectioned material. This may probably result from the small dimensions of the male fundaments which make the chances of their complete appearance in sectioned material rather remote. It is probably for this reason that MURPHY, whose account is based largely on examinations of microtome sections, missed the stage or failed to see a complete unpierced antheridium.

The oogonial incepts arise either on the same hypha which gave origin to the antheridium or on distinct hyphae. The entry of such an oogonial incept into the male gametangium is shown in figure 3. In this early stage of penetration the oogonial incept is seen to be almost uniform in diameter except for a constriction at the antheridial wall. This constriction is a significant indication of the actual piercing of the antheridium, a point about which ROSE and LINDEGREN (10) and COOPER and PORTER (3) were doubtful. The hyphal region of the incept in the immediate vicinity of the antheridial wall is often swollen and congested owing to the tension set up within it as a result of the penetration. A thickening of the antheridial wall is initiated only upon the entry of the female incept into the an-



FIGS. 1–16.—Fig. 1, antheridial fundaments: *a*, lateral; *b*, terminal. Fig. 2, mature antheridium in binucleate condition. Secondary wall not yet laid down. Fig. 3, entry of oogonial incept into mature gametangium. Laying-down of secondary antheridial wall is initiated upon penetration of male by female gametangium. Deposition of this wall proceeds from apex downward. Antheridium is four-nucleate. Fig. 4, *A*, swelling of female incept just prior to its emergence from antheridium; *B*, gametangia shown in 4*A* as seen in another plane of focus, showing an antheridial papilla. Fig. 5, early stage of emergence of oogonium showing linear nuclei in oogonial cytoplasm with incipient stratifications. Fig. 6, early stage, as in Fig. 5, with spherical nuclei in nonstratified cytoplasm. Figs. 7–12, various stages in oogonial development showing association of linear nuclei with stratifications. Linear nuclei occur at stipe end of oogonium; spherical nuclei at distal end. Figs. 13–15, nuclear conditions at various stages after attainment of mature dimensions by oogonium. Fig. 16, nuclear division. Faint chromatin reticulum is seen in nuclei just about to divide. Chromosomes are short, stublike.

theridium. The thickening begins at the apex and extends downward to the base of the antheridium (fig. 3). A swelling of the oogonial incept occurs even before it has completely emerged from the antheridium (figs. 4*A*, 5).

Short, tubular processes formed by the antheridia of *P. erythroseptica* have been described by MURPHY. These he regarded as a means to accommodate the pressure developed within the antheridium as a result of oogonial entry. Large numbers of similar antheridial papillae have also been seen in *P. himalayensis* (figs. 4*B*, 14, 21, 22, 23, 28). The present observations do not substantiate MURPHY's conclusion as to their function. Rather, the writer is inclined to believe that these papillae are involved in fertilization. A fuller discussion of antheridial papillae appears later.

FURTHER DEVELOPMENT OF GAMETANGIA, AND NUCLEAR BEHAVIOR DURING COPULATION.—Growth of the oogonial incept beyond the stage illustrated in figure 4*A* is rapid, and the oogonium may assume the dimensions of the mature body within 3–5 hours. The cytoplasm, which in the beginning was granular and without vacuoles, gradually becomes clearer, and vacuoles make their appearance in keeping with the rapid increase in size of the oogonium.

Essentially, nuclear behavior both in the antheridium and in the developing oogonium follows a similar sequence of events. In both organs there occurs a division of nuclei followed by a degeneration. In this degeneration only one nucleus from the oogonium and one from the antheridium survive to function in fertilization.

The antheridium about to be penetrated by the oogonial incept possesses two large spherical nuclei (fig. 12). A division of these nuclei is initiated upon entry of the female incept. In the earlier stages of growth of the oogonium the number of nuclei in the antheridium is four. During the further growth of the oogonium a series of divisions of the antheridial nuclei may result in the formation of as many as twelve. It is curious that in the development of this multinucleate stage the nuclei tend to diminish in size—a phenomenon not figured by MURPHY for *P. erythroseptica*.

A phenomenon in nuclear behavior unique in the Oomycetae was first observed by MURPHY in *P. erythroseptica*. He described and illustrated a stage in development of the oogonium with stratified cytoplasm in which the nuclei are bow-shaped or linear and oriented tangentially along the arcs of concentric circles whose center lies somewhere in the oogonial stipe. These concentric arcs result from differences in density of the oogonial cytoplasm. A similar condition has been observed in *P. himalayensis* (figs. 10, 11). In this fungus, as in *P. erythroseptica*, the cytoplasmic stratifications gradually thin out toward the apex of the oogonium where the cytoplasm is vacuolate and homogeneous and without any evidence of alterations in density. The stratification phenomenon can be seen in even early stages of emergence of the oogonium from the antheridium (figs. 5, 7, 8, and 9), though at these stages the stratifications are not very pronounced. The phenomenon is most prominent when the oogonium assumes mature dimensions.

At this mature stage only the nuclei at the stipe end of the oogonium are linear or bow-shaped and are arranged along the cytoplasmic arcs, while those at the distal end of the oogonium (at its periphery) are spherical. According to MURPHY, these spherical nuclei result from a transformation of the proximal,

linear nuclei. MURPHY's belief is very probably unwarranted. He stated: "The nuclei, particularly in the oogonium, are linear for the most part." He was of the opinion that "they multiply mitotically in the hyphae about to form an oogonium." In support of his observation, he drew attention to his figure 13 of plate 2; but he has drawn other figures showing oogonia in earlier stages with spherical nuclei.

An alternative explanation for the occurrence of linear nuclei is favored here. In *P. himalayensis* the oogonial incept in its earliest stage of penetration has only a single nucleus (fig. 3). The linear nucleus has been seen to be invariably associated with the cytoplasmic stratifications, and this fact is significant. Judging from MURPHY's descriptions and diagrams, he also saw the association of the linear nuclei with cytoplasmic stratifications. It is important to note that spherical nuclei are not seen in these stratifications and that they are always seen at the distal end of the oogonium at its periphery. Therefore, it is probable that the linear form of the oogonial nuclei results from the pressure exerted on them owing to their positions in the stratified region of the cytoplasm. For, according to MURPHY, "one has the impression that it [cytoplasm] welled into the organ in waves or pulses, every wave being flattened out by the succeeding one and made visible by slight differences in density."

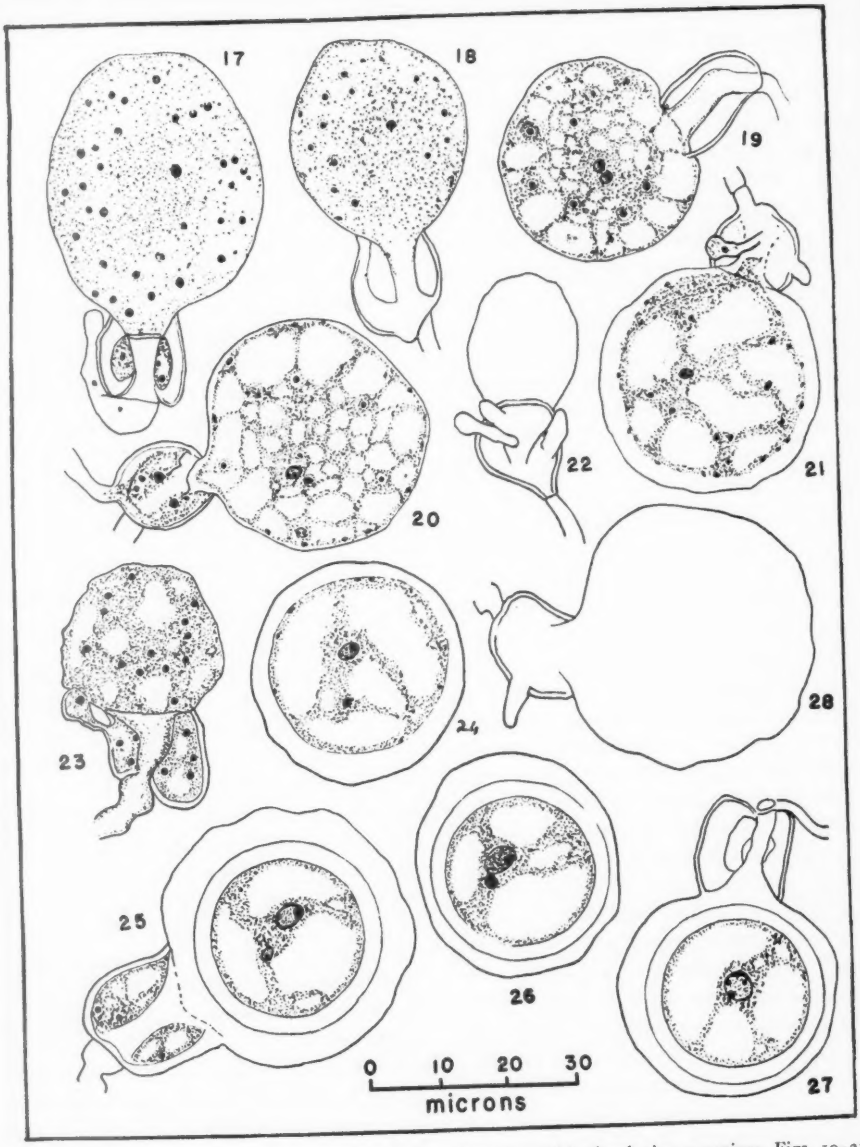
A striking feature in nuclear behavior in early stages of oogonial emergence is the symmetrical orientation of the elongate nuclei (figs. 7, 8). They are disposed in pairs along the long axis of the oogonium, and continued fission may result in three or four or possibly more pairs. At later stages (figs. 9–11) the nuclei disperse from the paired positions and occur wider apart from each other. The fate of the elongated nuclei could not be followed beyond the stage in which the cytoplasm has stratifications.

The number of nuclei in an oogonium is very variable. As many as forty occur in *P. himalayensis;* larger numbers, ninety to one hundred, have been reported in *P. erythroseptica.*

NUCLEAR DEGENERATION.—As in other Peronosporaceae, not all the nuclei that enter the oogonium of *P. himalayensis* are destined to be fertilized and to take part in oospore formation. Of the twenty to forty oogonial nuclei, all but one degenerate and are eventually absorbed into the cytoplasm, leaving behind a solitary privileged female nucleus to participate in fertilization.

Nuclear-degeneration figures in *P. himalayensis* are much the same as those reported in *P. erythroseptica:* "A deep stained undifferentiated sphere, larger than a nucleolus and smaller than a nucleus, surrounded by a clear halo without a trace of nuclear membrane" (figs. 18, 19). A migration of the oogonial nuclei toward the periphery and their degeneration are concomitant processes. The nuclei progressively become smaller as they migrate to the oogonial wall, where in late stages they disappear from view. A nuclear degeneration occurs in the antheridium at almost the same time. Halos around degenerating male nuclei, however, have not been observed in *P. himalayensis.*

In the oogonium not all nuclei degenerate, however, in the process of their migration toward the periphery. A few still remain over, and, after an increase in size (as in *P. erythroseptica*), these undergo a mitotic division; the resulting nuclei, with the exception of one, are also ultimately absorbed into the oogonial cytoplasm. The course of events within the



FIGS. 17–28.—Figs. 17, 18, degeneration and migration of nuclei in developing oogonium. Figs. 19–21, further stages in degeneration of supernumerary nuclei. Division of central oogonial nucleus is seen in fig. 19. One of daughter nuclei degenerates; other is functional female nucleus. Degenerating daughter nucleus is seen in fig. 20. Figs. 22, 28, antheridial papillae. Fig. 23, antheridial papilla functioning as fertilization tube. Fig. 24, oosphere stage. Male and female nuclei lie in cytoplasmic bridges. Fig. 25, both male and female nuclei are larger. Fig. 26, fertilization. Fig. 27, oospore.

antheridium is rather irregular compared with that within the oogonium. In the antheridium the number of male nuclei that degenerate is very variable, and the time during which this occurs need not always coincide with that during which the oogonial nuclei degenerate. Thus, practically all male nuclei may have disappeared before the oogonial nuclei have migrated to the periphery (fig. 21), or they may persist up to stages as advanced as those in which the oospore walls have been laid down (fig. 25).

NUCLEAR DIVISION.—In nuclei so small as those of *P. himalayensis* it is difficult to make observations of nuclear figures during division with the same precision as that possible with the nuclei of higher plants. In the few dividing nuclei favorably stained with haematoxylin the following was observed: Prior to division the nucleolus is quite large, spherical, and prominent. As the time for division approaches, the nucleolus loses its definite structure, and a faint chromatin reticulum is seen. Centrosomes have not been seen in this fungus during any stage of nuclear division. The chromosomes are short, are stublike, and are three in number in the haplophase. Prophase, metaphase, etc., positions could not be clearly discerned.

CYTOPLASMIC ALTERATIONS DURING NUCLEAR CHANGES.—The oogonial cytoplasm in the beginning completely fills the oogonial cavity, is granular, and stains deeply with safranin. It later undergoes alterations in consistency. Vacuoles begin to appear, and these may occupy the greater portion of the oogonial cytoplasm. Large vacuoles are first seen at the time of mitotic division. The cytoplasm, because of the vacuoles, is highly reticulate and forms a thin ring on the inside of the oogonial wall. Degenerating nuclei occur in strands of cytoplasm, and large numbers are found at the periphery. By the time most of the oogonial nuclei have degenerated, the cytoplasm becomes differentiated into a clear, hyaline, structureless periplasm surrounding a central ooplasm (figs. 16, 20, 21). Unlike that of *Albugo*, however, the periplasm is very small in extent and is in the form of a thin layer. In some cases where the ooplasm has contracted from the wall, it may be thicker (fig. 21).

THE OOSPHERE AND FERTILIZATION

As in *P. erythroseptica* there occurs in *P. himalayensis* a division of the central privileged nucleus while the supernumerary nuclei are disintegrating (figs. 18, 19). One of the daughter nuclei resulting from division of the privileged nucleus also diminishes in size and is eventually absorbed (figs. 20, 21). The other daughter nucleus is functional and constitutes the egg nucleus.

In the beginning the egg nucleus is spherical and no larger in size than the other oogonial nuclei prior to their degeneration. With a progressive contraction of the cytoplasm from the oogonial wall to form the oosphere, there occurs a gradual enlargement of the young egg nucleus. It loses its spherical form and becomes elliptical (figs. 25, 26). At the same time there occur changes in the consistency and vacuolation of the cytoplasm. The latter becomes clearer, and eventually no granules are to be found. Vacuolation may become more prominent and may occupy the greater part of the developing oosphere, holding the egg nucleus at the confluence of cytoplasmic bridges (figs. 20, 24, 25). The remnants of the degenerated nuclei are found at this stage at the periphery of the ooplasm. MURPHY reported that in *P. erythroseptica* these remnants form a diffusely staining, almost continuous line

separating the ooplasm from the periplasm. Such a condition has not been observed in *P. himalayensis*.

By this time the oogonial cytoplasm has contracted considerably from the wall, and the laying-down of the primary membrane is initiated. The deposition of this membrane (the "primitive wall" of STEVENS) coincides with the complete absorption of the scanty periplasm and the development of the typical oosphere stage.

Fertilization in *P. himalayensis* presents certain unique features not previously recorded in other *Phytophthora* species. MURPHY, in his account of *P. erythroseptica*, described in considerable detail the structure and significance of a "manocyst" or receptive papilla protruding from the stalk of the oogonium into the antheridium. The appearance of the manocyst, which is later withdrawn, is, MURPHY reported, the prelude to the formation of a short fertilization tube growing in at the same place and delivering one male nucleus and the greater part of the antheridial cytoplasm into the oogonium.

In spite of a thorough search for such structures in several hundreds of copulating gametangia, manocysts have not been seen in *P. himalayensis*. The manocyst and fertilization tube are, according to MURPHY, so conspicuous that it is doubtful if one has missed seeing them in *P. himalayensis*, especially as they have been reported to be not ephemeral. For, according to MURPHY, a vestige of the manocyst is still evident after it is withdrawn. It may be supposed that in *P. himalayensis* a male nucleus might enter the oogonium through an opening made by the dissolution of the intervening walls without the occurrence of a special structure like the receptive papilla or a fertilization tube as described by MURPHY. Such a transfer of the male nucleus has not, however, been observed in this study.

If it is argued that the male nucleus may enter the oogonium at the stage when the oogonial incept is within the antheridium, then there must always be two functional nuclei, the male and the female, until the two fuse with each other at a later stage; but, as we have seen above, there is at a particular stage only one nucleus and this is the female nucleus, with a degenerating daughter nucleus and a number of degenerating supernumerary nuclei. On the other hand, fertilization as seen in many gametangia is of much interest.

On previous pages, in a discussion of the ontogeny of *P. himalayensis*, attention has been called to the development of short papillae by the antheridium. Such structures have also been reported in *P. erythroseptica*. MURPHY postulated that these tubes of short growth function in easing the pressure developed within an antheridium as a result of entry and growth of the oogonial incept in it. The present study does not support such a view. On the basis of MURPHY's explanation one would expect pressure to be greatest at a time when the oogonial incept is swelling within the antheridium just before its emergence (fig. 4). Consequently, antheridial papillae would be expected to appear at this stage. This, however, is not the case. In the majority of cases seen, papillae had developed at later stages.

My observations indicate that these antheridial papillae are associated with the fertilization process in *P. himalayensis*. The protrusions are seen to best advantage in material stained and mounted whole. Unlike the manocyst and fertilization tube of *P. erythroseptica* which appear at a definite stage of growth and

nuclear condition of the oogonium, the antheridial papillae of *P. himalayensis* may develop at any stage; thus, they may develop even when the oogonial incept is almost wholly within the antheridium (fig. 4*B*), or later, at various stages of oogonial development. Whenever a nucleus has been seen in the papillae, it has been observed that only one papilla has the nucleus in it. This nucleus is the male one. In the case of antheridia developing two or three papillae only one functions in the fertilization process. The others are often found emptied of their protoplasmic contents following a transfer of antheridial protoplasm into the oogonium through a papilla. The papilla grows upward from the amphigynous antheridium toward the oogonium and behaves much as the paragynous antheridium of other species of *Phytophthora*. The apex of this papilla is swollen and appressed closely to the side of the oogonium. A depression of the oogonial wall is found at the point of contact of the papilla with the oogonium. The papillae are double walled and with granular, deep-staining cytoplasm and a single nucleus (figs. 21, 23).

Owing to the origin of such papillae from amphigynous antheridia and their homology with paragynous antheridia, such a type of gametangial copulation demonstrates the fundamental similarity of paragyny and amphigyny.

An actual entry of the male nucleus into the female gametangium has not been seen, but a distinct dissolution of the walls intervening between the papilla tip and the oogonial wall, resulting in plasmogamy, has been observed. No evidence for an entry of an antheridial nucleus into the oogonium by any other means exists. There can be no doubt, therefore, that this nucleus within the papilla is the functional male nucleus. Fertilization of the female nucleus may or may not occur at the oosphere stage. In general, as in *P. erythroseptica*, fertilization may be postponed even until after the oospore walls have been laid down (fig. 26). Prior to caryogamy, the male and female nuclei come to occupy positions at some distance from one another along the cytoplasmic bridges and persist thus while the oospore walls are being deposited.

During this time both male and female nuclei increase in size, the male nucleus remaining spherical and the female becoming elliptical. Nucleoli are conspicuous in both nuclei and occur as intensely stained organelles. Eventually, the male and female nuclei come to lie side by side, and there occurs a flow of nuclear material from the male into the considerably larger female nucleus.

The antheridium is, as a rule, completely emptied of its contents; but cases where some cytoplasm and three or four nuclei persist even at this stage are not infrequent (fig. 25). The oospore, as in *P. erythroseptica*, is smooth walled and consists of three layers: (*a*) the primitive wall (the former oosphere periphery); (*b*) the primary endospore, a thin hyaline layer; and (*c*) the thick, outer, secondary endospore.

Summary

1. The morphology and cytology of the sex organs of a new species of *Phytophthora*, *P. himalayensis* Dastur, have been studied and compared with those of *P. erythroseptica*, the only other amphigynous *Phytophthora* investigated so far.

2. Accounts of some workers, who claimed that the amphigynous relation of the sex organs is an illusion resulting from an encircling of the male around the female gametangium, are considered, and the view is held here that the oogonial in-

cept does actually penetrate the antheridium.

3. Nuclear behavior in the male and female sex organs is described from the earliest stages to the formation of the oospore. The antheridial nuclei are few, being generally four, sometimes seven or eight. The oogonial nuclei are variable in number; not more than sixty have been seen in one oogonium. In both organs a degeneration of nuclei occurs during which only one nucleus from the oogonium and one (generally) from the antheridium survive. These privileged nuclei function in fertilization. The elongate form of oogonial nuclei occurring at a particular stage in development of the oogonium is regarded as resulting from the pressure exerted on them by the cytoplasm which assumes stratifications at this stage. A detailed study of chromosome behavior could not be made, owing to the small size of the nuclei.

4. A manocyst has not been seen in *P. himalayensis;* antheridial papillae recorded by MURPHY in *P. erythroseptica* have also been observed in *P. himalayensis*. My studies, however, do not substantiate MURPHY's conclusion as to their function; viz., that they serve to accommodate the pressure developed within the antheridium as a result of oogonial penetration.

5. A unique type of copulation, in which the antheridial papillae are involved, is described. Owing to the origin of such papillae from amphigynous antheridia and their homology with paragynous antheridia, such a type of gametangial copulation demonstrates the fundamental similarity of paragyny and amphigyny.

This work was done at the Indian Agricultural Research Institute, New Delhi, and I am indebted to Professor J. F. DASTUR for much help rendered during its course. I am also thankful to Dr. C. M. TUCKER of the University of Missouri for a criticism of this article.

SOUTHERN ILLINOIS UNIVERSITY
CARBONDALE, ILLINOIS

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GAMETOGENESIS AND OOSPORE FORMATION IN CYSTOPUS (ALBUGO) EVOLVULI

M. J. THIRUMALACHAR, MARVIN D. WHITEHEAD, AND JOHN S. BOYLE

The white rust, *Cystopus evolvuli* Damle, on *Evolvulus alsinoides* L., a common prostrate herb, was described by DAMLE (2) from material collected in India. He stressed the occurrence of confluent oogonial and oospore walls, similar to the condition present in the genus *Sclerospora*, as an important diagnostic character. Gametogenesis and oospore formation of this fungus are reported in the present paper.

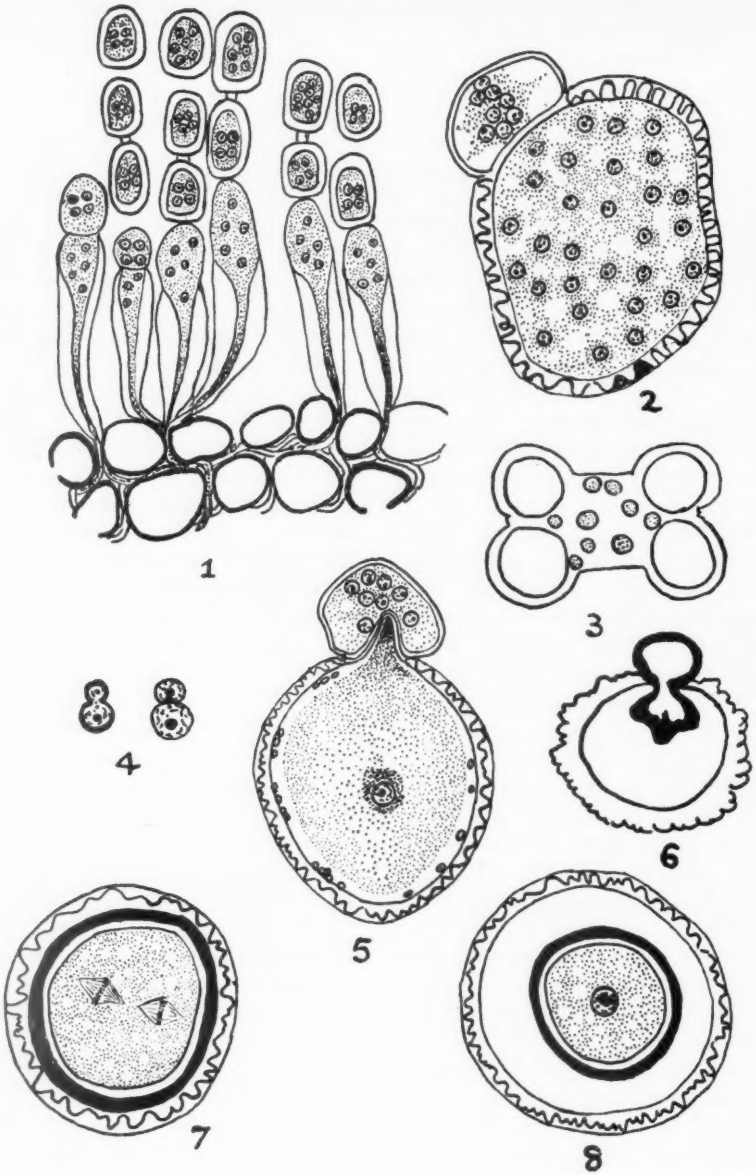
Characteristic symptoms of this white rust are the numerous sori, on leaves and shoots, which erupt and scatter white masses of sporangia. The sori at first are subepidermal, appearing externally as white spots, later becoming erumpent (fig. 1). Mature sporangia are subglobose to spherical without apparent equatorial thickenings. In case of severe infection, several of the developing shoots assume an erect habit of growth. This change from prostrate to erect growth was noticed by DAMLE and was reported by BUTLER (1) in *Ipomoea reniformis* Chois. infected by *Cystopus* species. Infected flowers in which the oospores are formed show no hypertrophy.

Materials for study were collected near Bangalore, South India, and were fixed in Karpechenko's modification of Navaschin's fluid and in formalin–acetic alcohol. Microtome sections 6–10 μ thick were stained with Heidenhain's iron-alum haematoxylin and Newton's iodine gentian-violet. The oospores were germinated on films of water condensed on slides by following the method described by germinating rust teliospores (7).

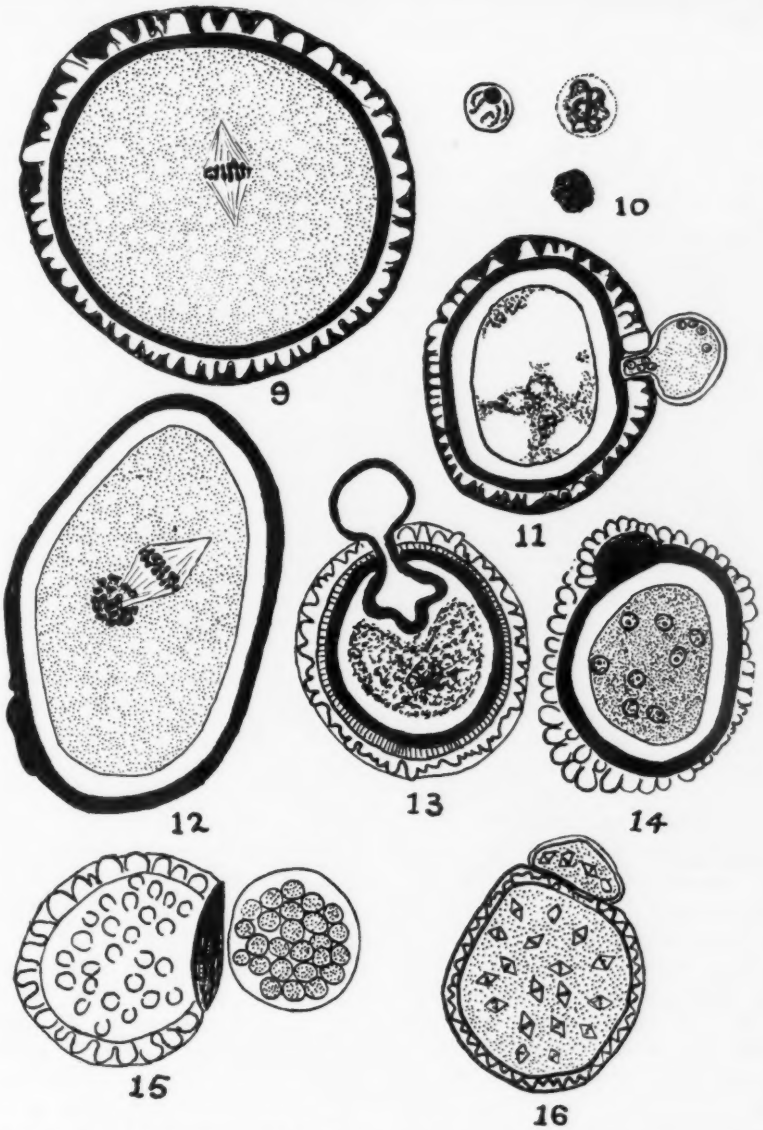
The sex organs of the fungus are formed within the floral parts and were never observed in leaf or stem tissues. Many infected flowers become virescent and appear abnormal. Microscopic examination revealed that oogonia and oospores are formed (fig. 3) in the wall of the anthers and in the tissues of the filaments, yet the pollen grains develop normally within the locules. Sections of the integuments and the nucellar tissues of the ovary showed oospores abundantly. The development of the embryo sac in infected flowers procedes to the eight-nucleate stage, after which degeneration sets in. In some severely infected flowers the brown mass of oospores fills the ovary.

The developmental stages of the fungus were studied in sections of the diseased tissues. Intercellular coenocytic mycelium develops irregular branches bearing the sex organs. In their early stages the antheridia and oogonia are multinucleate with the nuclei distributed uniformly in the cytoplasm (fig. 2). The wall of the oogonium is 3.5–5 μ thick and stains deeply. In the organization of the oosphere simultaneous nuclear divisions occur in both the antheridium and the oogonium (fig. 16). The dividing nuclei show no zonation characteristic of some of the other species of *Cystopus*. Further development is as in *C. candidus* (Pers.) Lev., with the differentiation of the ooplasm and periplasm following the migration of supernumerary nuclei toward the periphery. The mature oogonium is uninucleate with a deeply staining coenocentrum.

After the organization of the oosphere



FIGS. 1–8.—Fig. 1, section through sorus of *Cystopus evolvuli* showing sporangia. ×800. Fig. 2, young oogonium and antheridium. ×900. Fig. 3, section through anther of *Evolvulus alsinoides* showing oospores within connective tissue. ×100. Fig. 4, fusion of male nuclei with egg nuclei. ×1800. Fig. 5, receptive papilla and coenocentrum of oogonium. ×900. Fig. 6, mature oospore with remnants of antheridium. ×450. Fig. 7, second nuclear division of fusion nucleus. ×900. Fig. 8, young oospore. ×900.



FIGS. 9–16.—Fig. 9, first division of fusion nucleus. ×1800. Fig. 10, some stages in nuclear division. ×1800. Fig. 11, parthenogenetic development. ×900. Fig. 12, early stage in second division of fusion nucleus. ×1800. Fig. 13, same as fig. 6. ×900. Fig. 14, mature oospore showing eight nuclei. ×900. Fig. 15, germinating oospore. ×750. Fig. 16, simultaneous free nuclear divisions in antheridium and oogonium. ×750.

the portion of the ooplasm in contact with the antheridium bulges into the antheridium, which is in close juxtaposition, and forms the receptive papilla (fig. 5). This is followed by the entry of the fertilization tube from the antheridium, from which a single male nucleus enters and fuses with the egg nucleus (fig. 4). The antheridium and the fertilization tube persist after fertilization, and the remnants of the antheridium may be observed in most of the mature oospores (fig. 6).

The differentiation of the wall of the oospore is evident soon after fertilization (fig. 8). The wall comprises two thin layers at first which thicken gradually and become confluent with the oogonial wall, similar to the condition present in *Sclerospora* (figs. 13, 14). The oospore wall is smooth in contrast to the wrinkled nature of the oogonial wall. Consequently, the mature oospores appear wrinkled or tuberculate in surface view. Parthenogenetic development of oospores was observed in a few cases in which the fertilization tube had failed to penetrate the oogonial wall before the oospore wall developed (fig. 11).

The mature oospores usually contain eight, rarely sixteen, nuclei (fig. 14). These originate from the three, rarely four, successive free nuclear divisions of the fusion nucleus. The exact stage at which meiosis occurs in the life-cycle of *Cystopus* has been a point of contest by different investigators. WAGER (9) thought that the reduction division occurred just prior to the formation of zoospores during oospore germination. STEVENS (5), in his early studies on *Cystopus blitii* (Biv.) de Bary, assumed that meiosis took place during the first two divisions of the nuclei in the developing oogonium. TSANG (8), working on *C. candidus* and *C. tragopogonis* (Pers.) Schr., reported twenty-four chromosomes at the metaphase plate of the first division of the fusion nucleus and twelve at the second anaphase division. FITZPATRICK (3) wrote that there is much reason to regard the two simultaneous mitoses which occur in the gametangia preceding fertilization as reducing divisions or, from analogy with other fungi, that the first two mitoses of the fusion nucleus may well be regarded as accomplishing reduction. Some of the observations made on the divisions of the fusion nucleus in *C. evolvuli* are presented here.

Prior to undergoing divisions the fusion nucleus enlarges slightly in size, manifesting a large nucleolus and a fine network of chromatin reticulum (fig. 10). The differentiation of chromosomes as spirally banded structures marks the prophase (fig. 10). The chromosomes organize at the equatorial plate with the formation of spindles (fig. 9). The nuclear membrane is inconspicuous during late metaphase stages. Synapsis or diakinesis was not followed. Chromosome counts made at the metaphase stage of the first division of the fusion nucleus showed about sixteen chromosomes. The small size of the chromosomes prevented more accurate counting. The daughter chromosomes are translocated to the opposite poles and there organized into daughter nuclei. Sometimes the second division takes place even without an interphase following the first division. The spindles of the two dividing daughter nuclei are either parallel or at right angles to each other (figs. 7, 12). Chromosome counts made in the polar view of the metaphase plate revealed about sixteen chromosomes. In three instances the late anaphase stages of the second division of the fusion nucleus were observed. In polar view seven to eight chromosomes were

counted, indicating that chromosome reduction had occurred.

The oospores were germinated by the method already described. The exospore ruptures and the endospore, inclosing the zoospores, protrudes. In some germinating oospores the endospore inclosing the zoospore mass emerges completely from the outer spore-coat. (fig. 15)

Discussion

The study of gametogenesis and oospore formation of *C. evolvuli* has added further details on spore formation, fertilization, and chromosome reduction in this genus. As described by DAMLE (2), the confluent nature of the walls of the oogonium and oospore resemble those of *Sclerospora*. The thickening of the oogonial wall in *C. ipomoeae-panduranae* (Schw.) Stev. et Swingle was described by STEVENS (6) and ascribed as a condition reminiscent of *Sclerospora*. Recent studies on gametogenesis and oospore germination in *Sclerospora graminicola* (Sacc.) Schroet. by MCDONOUGH (4) revealed again several features common with *Cystopus*. In the present paper chromosome counts made during the first two divisions of the fusion nucleus of the young oospore indicated that reduction division takes place during the second division.

The writers wish to record their sense of gratefulness to Dr. JAMES G. DICKSON, University of Wisconsin, for valuable suggestions and kind interest.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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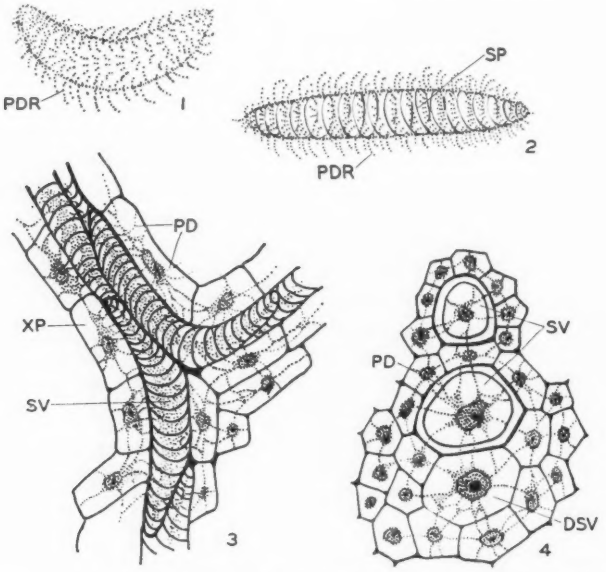
PLASMODESMATA IN XYLEM VESSELS

FLORA MURRAY SCOTT

Although it is generally accepted that intercellular protoplasmic connections are ubiquitous in plant tissues, so far as I am aware plasmodesmata have not been demonstrated previously in xylem vessels (**1**, **2**, **3**). One of the standard microchemical tests for cellulose—irrigation of sections with IKI followed by H_2SO_4 ($\pm 80\%$)—used in the study of cystoliths (**9**) and in the examination of the internal surface of leaves (**10**) proves equally effective in the study of vascular elements. Plasmodesmata are distinguishable in differentiating and mature vessels—spiral, reticulate, and pitted—throughout the plant and appear, for instance, in the leaf of *Acanthus mollis*, the stem of *Echinocystis macrocarpa*, and the root of *Vicia faba*.



FIGS. 1–4.—Figs. 1–3, *Acanthus mollis*, leaf. Fig. 1, protoplast of unlignified differentiating spiral vessel separated on destruction of cell wall in IKI–H_2SO_4, showing spiral arrangement of plasmodesmata. Fig. 2, protoplast of semilignified vessel, a later stage in differentiation, from same preparation, showing remains of partially lignified spiral thickening between projecting plasmodesmata. Fig. 3, paradermal section in midplane of leaf, showing spiral vessels at vein fork with plasmodesmata between adjacent vessels and parenchyma (approximate size of vessel segments $70 \times 15\,\mu$). Fig. 4, *Echinocystis macrocarpa*, young stem. Transection of part of vascular bundle, showing lignified and unlignified differentiating spiral vessels with plasmodesmata (approximate size of vessel segments $100 \times 50\,\mu$). *DSV*, differentiating spiral vessel; *PD*, plasmodesmata; *PDR*, remains of plasmodesmata; *SP*, spiral vessel segment; *SV*, spiral vessel; *XP*, xylem parenchyma.

In paradermal sections of the young leaf of *Acanthus*, one-fifth to one-fourth full size, spiral elements appear in the

developing veins. On addition of IKI the protoplasts are clearly visible. During irrigation with H_2SO_4, as the cellulose walls swell and turn blue, the intercellular connections become clearly defined and persist after total solution of the cellulose. If the material is completely macerated, as is readily done by tapping the cover glass during the irrigation process, the vessel segments and other cells are separated. The naked protoplasts of unlignified and semilignified spiral elements then more or less resemble microscopic polychaetes, with projections, the remains of plasmodesmata spirally (not segmentally) arranged, in place of parapodia (figs. 1–3). Irregularity in outline of protoplasts and nuclei in these and other drawings is the characteristic result seen during irrigation with H_2SO_4.

The bundles of *Echinocystis macrocarpa* previously described (**8**) provide excellent material for the demonstration of plasmodesmata in the stem. In the primary spiral vessels and also in the wider secondary pitted elements, intercellular protoplasmic connections appear between vessel and vessel and also between vessel and adjacent xylem parenchyma (figs. 4, 5).

In the classic and much investigated Windsor bean, *Vicia faba*, plasmodesmata are present in the xylem vessels of the growing root (figs. 6, 7). In vigorous seedlings about 15 cm. high, the longest secondary roots measure approximately 8 cm. in length. The xylem consists of typical elements—spiral, reticulate, and pitted vessel segments—which increase in diameter as they differentiate in normal sequence. Protoplasts with plasmodesmatal connections appear in differentiating spiral vessels near the root tip. They are also evident in the secondary pitted vessel segments, both differentiating and mature, at a distance of 8 cm. behind the growing point, about 5 cm. from the active root hair zone. The vessel segments measure from 100 to 200 μ in length, so that when sections of imbedded or frozen material are cut, as commonly, at a thickness of 10–15 μ, the cell contents completely disappear during the subsequent staining and mounting. This presumably is one reason for the current neglect of these living cells.

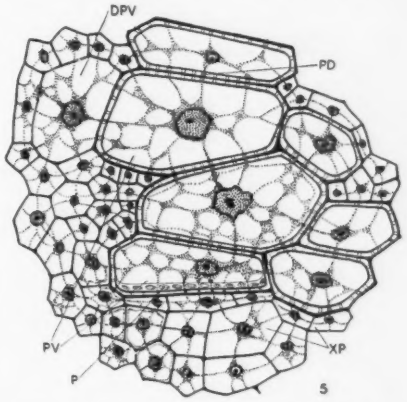
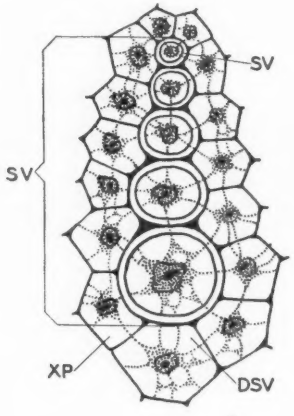


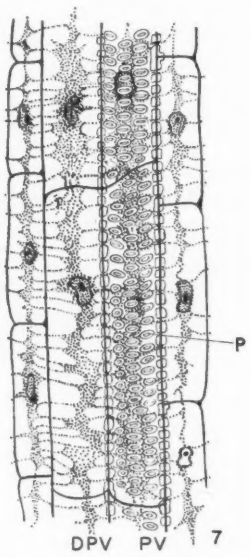
FIG. 5.—*Echinocystis macrocarpa*, older stem. Transection, showing lignified and unlignified differentiating pitted vessels with plasmodesmata (approximate size of vessel segments 300 × 200 μ). *DPV*, differentiating pitted vessel; *P*, pit; *PD*, plasmodesmata; *PV*, pitted vessel segment; *XP*, xylem parenchyma.

The ubiquitous occurrence of plasmodesmata is of undoubted significance in physiological problems connected with the movement of solutes in the plant. In some of his earlier papers, PRIESTLEY (**5, 6, 7**) discussed the function of the endodermis in relation to the entrance and transport of water in the root. In the seedlings of *Vicia faba* examined the xylem elements remain alive throughout at least 8 cm. of the root length. In this case, therefore, the problem of the passage of water from living parenchymatous cells to nonliving empty vessels does

not arise in the root hair zone but is necessarily relegated to a higher level in the vascular system. Moreover, as differentiation of primary and secondary vascular elements continues during the growing season, a network of variable thickness of living xylem elements presumably extends from the root upward into stem and leaves. That living cells cannot be ignored in the problem of solute movement is indicated in the comparatively recent statement of MEYER and ANDERSON (4) that "the influence of certain environmental factors upon absorption, particularly temperature and oxygen, suggest that the metabolic activities of living cells in the absorption zone of roots also play a role in this process." The protoplasts of *Vicia faba* afford material for the further study of this hypothesis.

It is also evident that cell walls cannot be considered as the physical equivalents of the homogeneous semipermeable membranes currently used in experiments on osmosis. Cell walls are heterogeneous perforate structures, the pores of which are filled with living protoplasmic strands presumably capable of active metabolism. Further investigation is required in order to determine whether the pattern of living xylem as here described is repeated generally in other vascular plants, or whether, as seems hardly probable, it is confined to the three species mentioned, *Acanthus mollis*, *Echinocystis macrocarpa*, and *Vicia faba*.





FIGS. 6, 7.—*Vicia faba*. Fig. 6, young root. Transection of part of primary xylem, showing several lignified, and one unlignified, differentiating spiral vessels with plasmodesmata (approximate size of vessel segments 100 × 15 μ). Fig. 7, older root. Longisection of part of secondary xylem, showing lignified pitted and unlignified differentiating pitted vessels with plasmodesmata (approximate size of vessel segments 228 × 28 μ). *DPV*, differentiating pitted vessel; *DSV*, differentiating spiral vessel; *P*, pit; *PV*, pitted vessel segment; *SV*, spiral vessel; *XP*, xylem parenchyma.

DEPARTMENT OF BOTANY
UNIVERSITY OF CALIFORNIA
LOS ANGELES, CALIFORNIA

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TRANSMISSION OF THE FLORAL STIMULUS IN SOYBEAN[1]

ARTHUR W. GALSTON

Introduction

Several years after their original (**13**) discovery of photoperiodism, GARNER and ALLARD (**14**) found that localization of the photoperiodic response may occur in *Cosmos*, a short-day plant. They also noted that if one part of the plant was kept completely in the dark and some other part above it was photoinduced to flower, then the part in the dark also flowered. This suggested that a mobile floral stimulus traveled from the illuminated to the darkened portions. Additional evidence of a mobile floral stimulus in various plant species has since then been obtained by numerous investigators (**3, 6, 7, 16, 19, 20, 21, 24, 25, 27**). The mobile stimulus, presumably a hormone, has been named "florigen" by CAJLACHJAN (**6**).

It naturally became of interest to determine the pathway of translocation of this stimulus in the plant. LUBIMENKO and BOUSLOVA (**24**) found that cutting the main vein of a single photoinduced leaf interrupted the transmission of the stimulus to the growing point. They therefore concluded that the floral stimulus is a chemical entity which may travel only in the vascular system. CAJLACHJAN (**10**) has reported that this is not true for *Perilla nankinensis*, where "florigen" may traverse leaf mesophyll, cortical parenchyma, and even the root. Inhibition of "florigen" transport by means of girdling (**9**), low temperatures (**5, 11**), narcotics (**11**), and scalding (**31**) would indicate that the floral stimulus is carried through living cells exclusively.

Various experimenters have shown that "florigen" may traverse a graft union between stems of donor and receptor plants (**8, 12, 16, 20, 25, 27, 28, 30**). In addition, reports of successful transmission of the floral stimulus from single donor leaves to receptor plants (**15, 17,**

[1] This work is part of a doctoral dissertation submitted at the University of Illinois in 1943 in partial fulfilment of the requirements for the Ph.D. degree. Certain of the experiments have been repeated and extended since that time.

18, 23) would seem clearly to establish the leaf as the locus of "florigen" synthesis.

Despite the comparative ease with which "florigen" may be transported throughout the plant, its existence outside the living plant cell has not yet been unequivocally demonstrated. Thus, whereas HAMNER and BONNER (16) reported transmission of "florigen" across a "diffusion contact" barrier of lens paper between the two graft partners, WITHROW and WITHROW (31) were later able to show that some tissue union occurs across such a barrier. With but few exceptions (2, 22, 29), attempts to extract florigenically active materials from plants have been unsuccessful. The only completely divergent result is that reported by MOSHKOV (27), who found that *Chrysanthemum* donor leaves could transmit the floral stimulus across a water gap to a vegetative receptor. Confirmation of this result would, in the present author's opinion, rule out the possibility that florigen may be merely a combination of mass nutrients and would definitely establish it as a water-soluble hormone, physiologically effective in micro-quantities.

The present work involves a reinvestigation of the transmission of the floral stimulus from a single grafted donor leaf to a receptor plant and an attempted repetition of the important "water-gap" experiment of MOSHKOV.

Material and methods

BORTHWICK and PARKER (4), studying the photoperiodic responses of twelve varieties of soybeans, were able to show that some varieties are of the short-day type, whereas others are indeterminate and will initiate flower primordia even under continuous illumination. The existence of great photoperiodic diversity within such a closely related group of plants seemed to recommend the use of soybeans as the experimental material in these studies.

Six-inch pots were used for growing the soybeans. Three seeds were planted, and two similar plants were allowed to develop in each pot. For long-day treatments, the natural day was prolonged to 17 hours by the use of 300-watt incandescent lamps placed about 1.5 meters above the bench on which the plants were grown. For short-day treatments plants were given natural illumination for 8 hours a day, being placed in a dark chamber at 4:00 P.M. and removed to the light bench at 8:00 A.M. Twelve pots were generally employed as one experimental group.

The technique used in making leaf grafts was that described by HEINZE *et al.* (18). When the plants were about 30 days old, a petiole of a half-expanded compound leaf of a receptor plant was cut obliquely, and the leaf-blade half was discarded. A donor leaf was then cut in the same way, and the distal portion was attached to the petiole end of the receptor leaf. The graft was then bound tightly with Parafilm, and the plant was transferred to a glass moist chamber for 7 days. During this week the grafted plants continued to receive the specific photoperiods being employed in the experiment. At the end of a week the grafted plants were removed to the greenhouse bench and later were examined for the presence of floral primordia. Macroscopic criteria were generally employed, but, before any plant was definitely classified as vegetative, the buds were dissected under a lens and examined for microscopic floral primordia.

Where petiolar scalding was employed in tracing the path of translocation of the

floral stimulus, donor petioles were scalded over a 2-mm. length 1 cm. from their cut end. This was done by wrapping a wisp of absorbent cotton around the area to be scalded, then carefully emitting a few drops of boiling water onto this area from the narrow nozzle of a wash bottle. The scalded petioles were then braced by portions of toothpicks and bound with Parafilm, as before. Although the number of successful grafts from such an operation was extremely small, the results are presented here for such informational value as they may have.

Results

PHOTOPERIODIC BEHAVIOR OF FOUR VARIETIES OF SOYBEANS.—According to BORTHWICK and PARKER (4), Biloxi and Peking soybeans will not flower if the photoperiod exceeds 16 hours, whereas Agate and Batorawka soybeans will flower even under continuous light. It seemed desirable to corroborate this behavior before proceeding with the other experiments.

Twelve pots of each variety were utilized for this experiment, six pots being exposed to each of the photoperiods listed in table 1. The plants were grown under this photoperiod from the time of emergence of the seedlings from the soil and were examined for the presence of floral primordia after 45 days. The results (table 1) agree with the observations of BORTHWICK and PARKER (4).

It seems clear that under a 17-hour light regime, Agate and Batorawka soybeans are capable of producing the floral stimulus, whereas Biloxi and Peking are not capable of so doing.

TRANSMISSION OF FLORAL STIMULUS BY LEAF GRAFTS.—HEINZ *et al.* (18) have shown that one leaf of Agate or Batorawka soybeans, grafted onto Biloxi receptors kept vegetative by a 17-hour photoperiod, can induce flowering over the entire plant. Four days of contact between leaf and receptor were found sufficient for transfer of the stimulus.

In the following experiments all plants were grown on a 17-hour photoperiod. When plants were 30 days old, leaf grafts were made as described above. One month later the plants were examined for presence of floral primordia. From the results (table 2) it is quite clear that both Agate and Batorawka donor leaves may transfer the floral stimulus to Peking and Biloxi receptors when all plants are grown under 17-hour photoperiods.

TABLE 1

PHOTOPERIODIC BEHAVIOR OF FOUR VARIETIES OF SOYBEANS

VARIETY	CONDITION OF PLANT AFTER 45 DAYS OF	
	8-hour day	17-hour day
Biloxi	Flowering	Vegetative
Peking	Flowering	Vegetative
Agate	Flowering	Flowering
Batorawka	Flowering	Flowering

On many of the unsuccessful grafts the donor leaves had apparently persisted on the receptor plant for the week that the plants spent in the moist chamber but had withered and died upon removal of the plant to the open greenhouse bench. These plants bore no floral primordia. In this one respect the data differ from those of Heinze *et al.*, who found 4 days of contact between donor and receptor sufficient to cause flowering.

PATHWAY OF TRANSLOCATION OF FLORAL STIMULUS.—Since translocation in the xylem may apparently proceed in the absence of any living cells, whereas translocation via the phloem is dependent on living cells, many investigators

have utilized the scalding technique for determining whether particular materials are translocated in the phloem or in the xylem. If the material in question is able to pass a scalded area, it is presumed to travel in the xylem; if it can be normally translocated but cannot pass a scalded area, it is presumed to travel in the phloem.

TABLE 2

INTERVARIETAL TRANSMISSION OF FLORAL STIMULUS BY MEANS OF LEAF GRAFTS

Receptor	Donor	No. of grafts attempted	No. of grafts successful	Condition of receptor	
				Fl.	Veg.
Peking	Agate	15	5	5	0
Peking	Batorawka	15	3	3	0
Biloxi	Agate	15	4	4	0
Biloxi	Batorawka	15	3	3	0
Peking	Peking (control)	10	4	0	4

With the technique described above scalding experiments were used to determine the pathway of transport of the floral stimulus from donor leaves to receptor petiole stumps. Twenty leaf grafts were made of Agate donors onto Peking receptors, all plants having been grown under 17-hour photoperiods. Ten of these grafts were kept as controls, and the other ten donor petioles were scalded. All grafted plants were transferred to a moist chamber for a week and then removed to a greenhouse bench. All received 17-hour photoperiods uninterruptedly. Although the numbers involved are quite small, the results (table 3) seem clearly to indicate that scalding prevents passage of the floral stimulus from the donor leaf to the receptor plant. This would corroborate previous work indicating that the phloem is the tissue involved in the transport of "florigen."

ATTEMPTED REPETITION OF MOSHKOV "WATER-GAP" EXPERIMENT.—Because of the critical importance of the MOSHKOV experiment, its repetition was attempted with the soybean material utilized in the previous experiments. As before, 17-hour photoperiods were utilized to keep the Peking receptors vegetative. Month-old plants were deprived of all leaves except the youngest compound leaf. The petiolar stump of the next oldest leaf was encased in a glass tube fastened onto its base by a split cork and held in place by a ring-stand and clamp. A similar split-cork arrangement at the upper end of the glass tube was provided for the attachment of a donor 17-hour Agate or Batorawka leaf. The glass tube was kept filled with distilled water, and leakage was prevented by coating the connection with petroleum jelly. Leaves were replaced and water added three times a day, this procedure continuing for 8 days. The length of the water gap was approximately 1 mm. The experimental setup is depicted in figure 1.

At the end of the 8-day experimental period the receptor petioles were starting to turn yellow. These petioles were therefore removed, the apparatus dismantled, and the receptor plants allowed to continue growth on a 17-hour photoperiod. Observation of axillary and terminal buds of plants so treated failed to reveal any traces of floral primordia 3 weeks after completion of the treatment.

TABLE 3

EFFECT OF PETIOLE SCALDING ON PASSAGE OF FLORAL STIMULUS

Receptor	Donor	Treatment	No. of successful grafts	No. of flowering receptors
Peking	Agate	Control	6	6
Peking	Agate	Scalded	2	0

In subsequent experiments the ratio of donor to receptor tissue was increased by placing numerous donor cuttings together with one receptor cutting into small shell vials containing a small volume of Hoagland's solution. All leaves except the youngest compound leaf were removed from the receptor, since mature leaves on an uninduced receptor reportedly (18) inhibit floral initiation. All flower buds on the donors were removed as they appeared, since "florigen" is apparently (21) used up by such buds. A 17-hour photoperiod was used throughout the experiment. Control vials contained only receptor Peking cuttings.

After having spent 10 days in the vials with the donors, the receptors were transferred to a sand rooting-bed for 2 weeks, then to soil in 6-inch pots. After one additional month the axillary and terminal buds were dissected. No floral primordia were found. Thus, the donor cuttings were unable to transmit their "florigen" to the receptor cuttings when separated from the latter by a brief water gap.

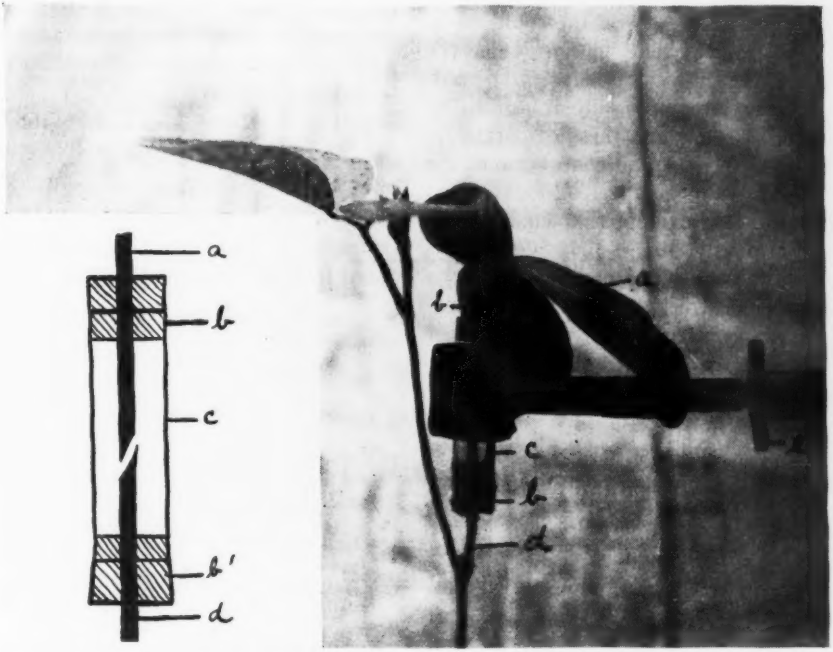

FIG. 1.—Attempted repetition of MOSHKOV's "water-gap" experiment. Diagram shows detail. *a*, donor leaf; *b*, *b*¹, split corks; *c*, glass tube; *d*, receptor plant; *e*, clamp.

Discussion

The data presented in this paper are in agreement with the previously held view that "florigen" is translocated by means of living cells only. The apparent failure of "florigen" to traverse a water gap from donor to receptor may indicate that (*a*) "florigen" is insoluble in water, (*b*) "florigen" is unstable outside the living cell, (*c*) insufficient quantities are transmitted to induce floral initiation, or (*d*) "florigen" cannot traverse the differ-

entially permeable membrane of the cell. The last possibility would imply that it normally moves from cell to cell through the plasmodesmata.

It is difficult to reconcile these findings with the results obtained by MOSHKOV (**28**) or with the occasional reports of florigenically active extracts obtained from plants. In view of the evidence (**1, 12, 18, 26, 28**) that "florigen" may be transmitted intervarietally, interspecifically, and intergenerically, it is probably not valid to explain these disparate results by appeal to differences between the kinds of plants used by various experimenters. It is evident that final acceptance of the hypothesis that "florigen" is a specific floral hormone must await the clear demonstration that active extracts may be prepared from photo-induced plants.

Summary

1. If Biloxi, Peking, Agate, and Batorawka soybeans are grown under a 17-hour photoperiod, the former two will remain vegetative, whereas the latter two will initiate floral primordia.

2. If single Agate or Batorawka leaves are grafted onto Peking or Biloxi receptors previously maintained vegetative by a 17-hour photoperiod, the receptors will be induced to flower.

3. The floral stimulus is incapable of passing from donor leaf to receptor petiole if the donor petiole is scalded.

4. The floral stimulus is incapable of traversing a 1-mm. water gap between donor leaf and receptor petiole.

KERCKHOFF LABORATORIES OF BIOLOGY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

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NOTES ON THE ANATOMY OF TWO CARBONIFEROUS PLANTS SPHENOPHYLLUM AND PSARONIUS

FREDDA D. REED

Introduction

The following observations and notes were made in a study of a number of calcareous nodules or coal balls collected from widely different regions. Some of the petrifactions came from Indiana, some from Illinois, and still others were found in Texas. Despite their wide geographic origin, the collector, the late Dr. A. C. Noé of the University of Chicago, said of them: "They are all from coal fields which are part of the Allegheny formation of the Pennsylvanian system." More precise information concerning the origin of some of the coal balls is not now available; therefore, these notes will be of little value to the coal geologist but are offered because of their contribution to the morphology and anatomy and possibly the habit of certain Carboniferous plants.

Observations

SPHENOPHYLLUM

Fragments of *Sphenophyllum* are frequently found, not only in Carboniferous shales, where they are preserved as impressions, but in coal balls as well, where they are found with cellular detail. Yet because the fragments consist almost invariably either of impressions of leaves without cellular structure or of the woody portions of stems and roots, there are more lacunae in our knowledge of the habit and anatomy of the entire plant body than of many of the genera of Carboniferous plants which are much less frequently encountered. *Sphenophyllum* is so imperfectly known that investigators are not agreed as to its habit. In the early restorations of Carboniferous landscapes it is illustrated as a floating plant. SCOTT (**6**) was inclined to the opinion

that it was a trailing, climbing plant; whereas, in the reconstruction of the "Carboniferous Swamp Forest" in the Chicago Natural History Museum *Sphenophyllum* is represented as a low growing herbaceous plant, living in the same kind of situation and having much the appearance of the aquatic *Hippuris* or a small tufted *Galium*. The following observations may throw more light on its structure and habit.

SPHENOPHYLLUM PLURIFOLIATUM.—The sections of figures 1, 3, 4, and 5 are from stems with different geographic origin but are regarded as representing developmental stages of the same species, *S. plurifoliatum* Will.

Figure 1 is a transverse section of a stem taken from near the tip at a level where primary tissues only are present. In this section the soft tissues adjacent to the xylem have disappeared, leaving the xylem strand free in a hollow cavity; this results in the displacement of the strand. In transverse section the primary xylem has the characteristic triangular pattern of the species in which the small protoxylem elements occupy the corners and the larger centripetally developed metaxylem elements make a solid core.

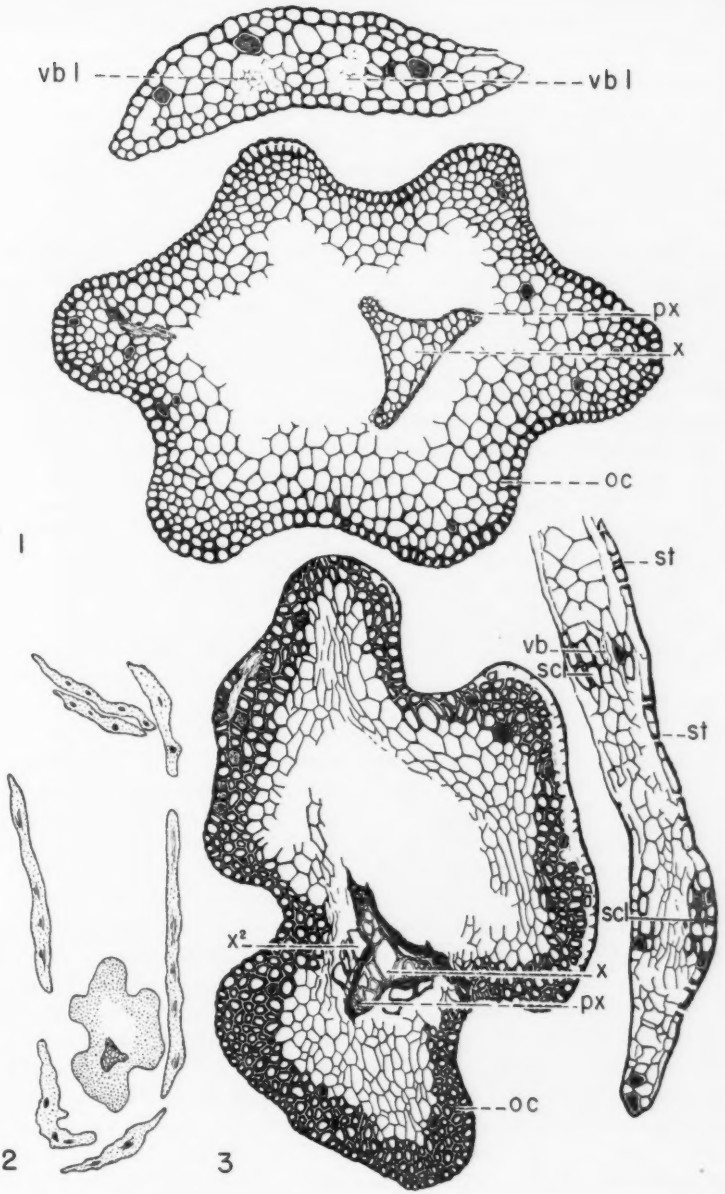
No phloem elements remain, nor is there any real or tangible evidence of its position and arrangement. An examination of the remaining tissues, however, indicates that the phloem was in three discrete strands alternating with the protoxylem. RENAULT (4) figured similar stems in which only primary tissues appeared; their organization was essentially the same as in this stem.

The inner cortex of the *Sphenophyllum* described by RENAULT was shown as having been composed of delicate, thin-walled tissue. That the inner cortex of the stem shown in figure 1 was of similar structure is indicated by its complete disappearance; being soft and delicate, it would not lend itself to preservation, whereas there is almost complete preservation of all thick-walled tissues.

The outer cortex varies in thickness from four to eight cells. These cells are relatively thick-walled and in optical section are roughly isodiametric. The smaller and thicker-walled cells are those adjacent to the epidermis, while from this hypodermal layer to the innermost layer which has been preserved the cells are progressively larger and progressively less thick-walled. The epidermal cells have walls as thick if not thicker than those of the hypodermis and appear to have formed a rigid layer. There are no evidences of stomata in this section.

The surface of young stems with little or no secondary increment is marked with longitudinal ridges which made the pattern of the transverse section roughly hexagonal (figs. 1, 3). There are three conspicuous furrows opposite the sides of the triangle of primary xylem, and three lesser ones opposite the protoxylem points.

A somewhat more mature region of a stem is shown in figure 3. Its more mature condition is demonstrated by the fact that it has some secondary xylem (fig. 3, X^2) and by the thicker walls and the generally mature aspect of the cortical cells. This stem, like that of figure 1, has lost its phloem and also the cells of the inner cortex; furthermore, the xylem strand shows evidence of having been crushed, and there is some displacement of the tissues. Nevertheless, the arrangement and distribution of the remaining cells demonstrate that the first secondary xylem elements to differentiate were those at the sides of the triangle of the primary xylem or between the phloem and the metaxylem. These patches of



FIGS. 1–3.—*Sphenophyllum plurifoliatum* Will. Fig. 1, transections of stem tip with primary tissues only and of one leaf: *px*, protoxylem; *x*, metaxylem; *oc*, outer cortex; *vb l*, vascular bundle of leaf. Fig. 2, diagram of transection of stem tip with some secondary wood and whorl of leaves. Fig. 3, detail of stem and portion of one leaf of fig. 2: X^2, secondary xylem; *st*, stoma; *scl*, sclerenchyma strands accompanying veins.

secondary xylem and those of the protoxylem are on alternate radii.

Although fragments of *Sphenophyllum* stems with cellular preservation are of frequent occurrence, young stem tips of the type described above are rarely encountered. Most of the stems found are comparable in degree of preservation and in size to that of figure 4. Preservation of this specimen is limited to the xylem region, which measures about 3 mm. in diameter. This stem has the usual triarch, exarch, protostelic organization of the primary xylem, the characteristic arrangement of fascicular and interfascicular secondary xylem elements, and the peculiar wood-ray formation of the species.

An unusual specimen of *S. plurifoliatum* is illustrated diagrammatically in figure 5. Its unusual features are its very large diameter and the presence of growth rings. The diameter is about 15 mm., which makes this *Sphenophyllum* stem one of the largest, if not the largest, on record. The xylem region is about 11 mm. in diameter. The growth rings are irregular and excentric, and the degree of size difference of the xylem elements is far more pronounced in some parts of the circumference of the ring than in others. A detail through a portion of one growth ring (fig. 6) shows the small secondary elements succeeding the larger ones. The cortex is poorly preserved, but there is indication of a well-developed and compact periderm.

These irregularities of growth are not regarded as being growth rings in the usual sense—that is, with the regular alternation of large and successively smaller xylem elements which is developed in response to periodic seasonal or climatic changes of temperature or rainfall. Instead they appear to have been formed as a result of some kind of interruption in the normal growth process such as might occur from wounding or more likely from a series of inundations.

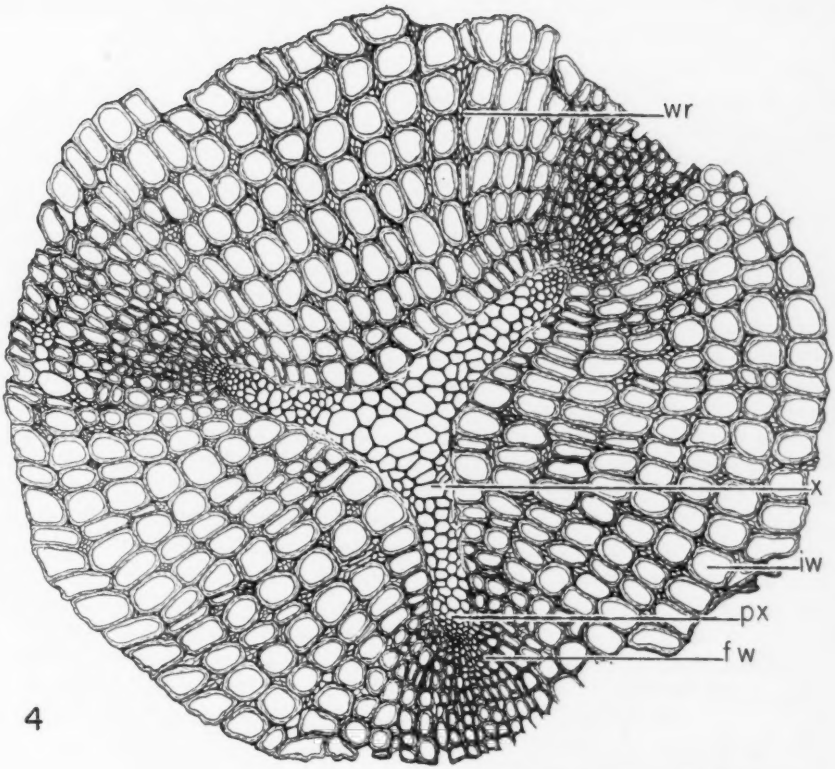
SPHENOPHYLLUM LEAVES.—In articles published in 1873 (3) and 1876 (4) RENAULT described and figured leaves of *Sphenophyllum*. From these investigations the anatomy of the leaf may be summarized briefly: the one to several (three to five) vascular strands are more or less parallel, and the bundles are apparently concentric. The tissue of the leaf was strengthened by bands of sclerenchyma lying next the epidermis of the upper and lower surfaces. The mesophyll appears uniform with no differentiation into palisade and spongy parenchyma.

RENAULT's figures have been repeatedly reproduced, but to my knowledge there have been no published investigations of *Sphenophyllum* leaves since that time.

The leaves herein figured are not so completely preserved as were those of the French specimens, but most of the tissues are observable, and it is possible to make a reconstruction of the anatomical pattern. Moreover, these specimens have the advantage of exhibiting different stages in maturation, thereby indicating something of their developmental sequence.

In figure 1 is shown a transverse section of a young leaf in which there is almost no differentiation of the cells. The vascular strands, of which there are two at this level, are indicated by lacunae (fig. 1, *vb l*). It would appear that there had been little or no lignification of vascular elements, and their cell walls, being yet soft, were not preserved.

Figure 2 is a diagrammatic representation of some older and apparently mature leaves in transection showing their relative position to one another and to the stem which bore them. A portion of a section of one of them (fig. 3) shows as



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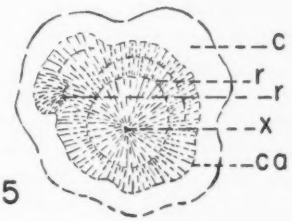

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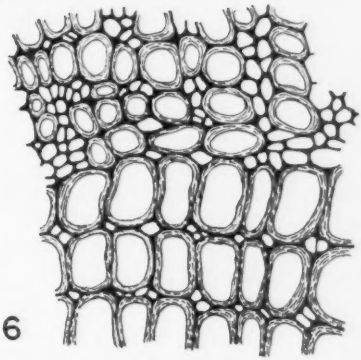

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FIGS. 4–6.—*Sphenophyllum plurifoliatum* Will. Fig. 4, transection of woody zone of mature stem: *fw*, fascicular wood; *iw*, interfascicular wood; *wr*, wood ray. Fig. 5, diagram of transection of stem showing irregular growth: *x*, primary xylem; *r*, "growth rings" of secondary xylem; *ca*, cambium; *c*, cortex. Fig. 6, detail through one of the "growth rings" of fig. 5; larger tracheids are toward center of stem.

much structural detail as the preservation admitted. These leaves are relatively thin, but they must have been strong, for both the upper and the lower epidermis are buttressed by strands of sclerenchyma which accompany the veins (fig. 3, *scl*). Again, the soft, thin-walled cells of the vascular strands have disappeared; therefore, the orientation of the elements or the type of bundle cannot be determined. There are some irregularities in arrangement of the cells of the lower epidermis which, from their position and distribution, indicate the presence of stomata (fig. 3, *st*). If these are stomata, then it is possible to write of them that they are numerous and that their guard cells are flush with the surface of the epidermis.

DISCUSSION.—A consideration of the anatomical structure of the *Sphenophyllum* fragments of both stems and leaves described above does not suggest an aquatic habitat for the genus. The well-developed and extensive vascular tissue, the presence of supporting tissue, and the absence of lacunar areas are all features indicative of a plant adapted to the land. Furthermore, most of the stem fragments that have been found possess an appreciable increment of secondary wood comparable to the one of figure 4; yet the leaves are found associated only with that region of the stem where there is little or no secondary xylem, and such specimens are rarely encountered. It would seem, then, that the stem of *Sphenophyllum* must have been long and slender, and the greater part of its length woody and leafless, with the leaves confined to the younger portions. Additional evidence that *Sphenophyllum* was a Carboniferous liana has been supplied by Dr. B. SAHNI,[1] who reported having found in India some impressions of stems bearing leaves in which the leaves of a whorl were unequal in size and arranged on the stem in a flat, dorsiventral pattern as if the plant had been trailing or scrambling over rocks or other vegetation.

[1] Personal communication.

PSARONIUS

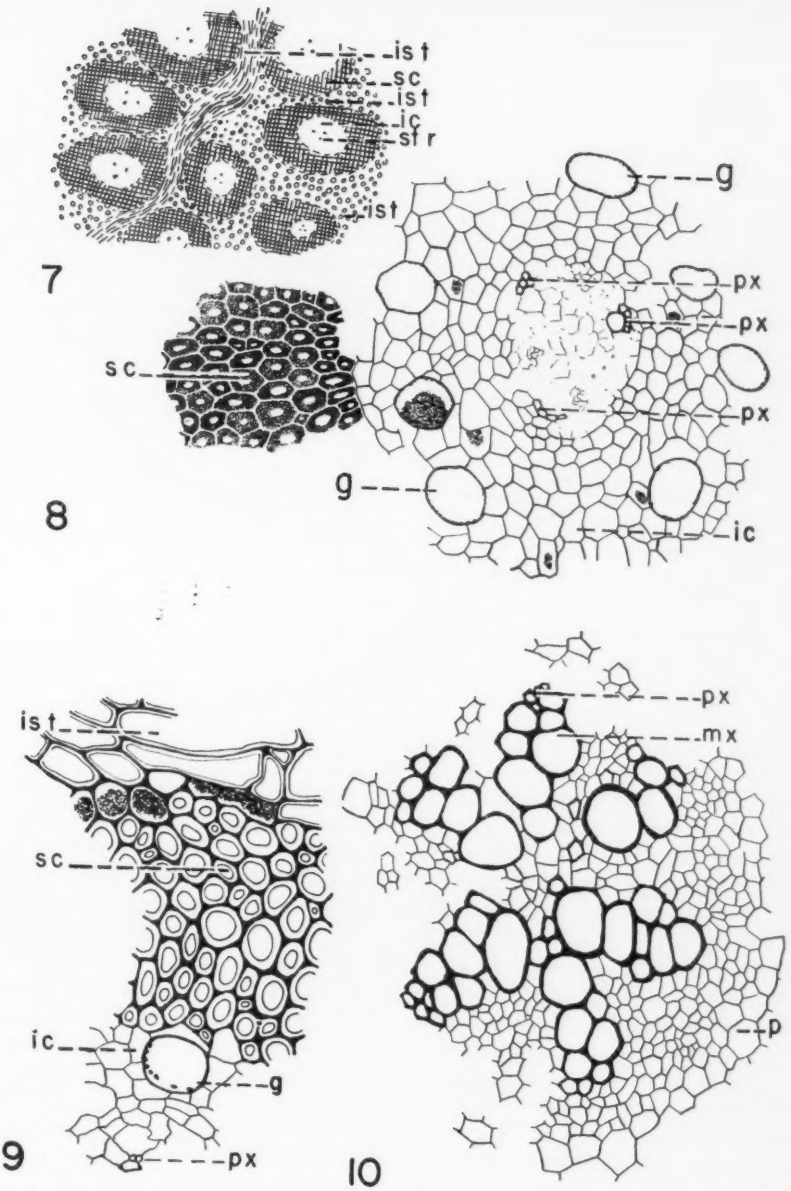
During the last century *Psaronius* Cotta was investigated frequently; however, it is beyond the scope of this paper to go into the history and literature, both of which have been reviewed by SEWARD (7), SCOTT (6), and, more recently, GILLETTE (1).

Although a number of species of *Psaronius* stems have been described from American strata, the frequency of stem fragments in coal balls is not so high as that of many other plant forms. This, however, may not be said of *Psaronius* rootlets, for they are almost as ubiquitous as are the rootlets of *Stigmaria*.

As is well known the stem was densely covered with adventitious rootlets which originated in the younger part and grew vertically downward. There were two distinct zones of rootlets: the upper, where the rootlets were small, crowded, and compact (fig. 7); and the lower zone, where they were larger and free from one another.

Figure 8 is a detail of a portion of a transection of a rootlet from figure 7. The rootlets of this specimen are triarch, and lignification is limited to the elements of the protoxylem. The cortex is differentiated in two regions. The cells of the inner region are thin-walled parenchyma; interspersed among them are some large elements which are thought to be gum canals (figs. 8, 9, *g*). Exterior to this zone is the outer cortex (fig. 8, *sc*). It is relatively massive and is composed of thick-walled sclerenchymatous cells.

The rootlets at this level are crowded



FIGS. 7–10.—*Psaronius* rootlets. Fig. 7, diagram of transection of portion of rootlet zone which surrounds stem: *is t*, interstitial tissue; *sc*, outer cortex of sclerotic tissue; *ic*, inner cortex; *st r*, stelar region. Fig. 8, transection of portion of rootlet of fig. 7: *px*, protoxylem; *g*, gum canal. Fig. 9, detail showing relation of outer cortex of rootlet to interstitial tissue. Fig. 10, stele of mature root: *mx*, metaxylem; *p*, phloem.

and appear to be organically connected by interstitial tissue (figs. 7, 9). The nature of the interstitial tissue has been argued, whether it is intra-cortical or extra-cortical—that is, whether it is made up in part of cortex of the stem through which the rootlets have intruded, or whether it is composed of a compact mass of filaments or hairs derived from the peripheral cells of the rootlet. SAHNI (5) has shown that both conditions may exist, and it would seem that one or the other might prevail depending on the level of the stem from which the section was taken.

The rootlets of the second zone—where they are free from one another—are larger. They vary considerably in diameter; those of one coal ball ranged from 0.2 to 1.5 cm., but most of them approximate 1.0 cm. The number of protoxylem points in the exarch protostele varys from three to seven (figs. 10, 12). There is the usual radial arrangement of phloem and protoxylem, and these tissues are succeeded in turn by pericycle and endodermis. The endodermis is not sharply differentiated in any of the rootlets examined, and in none of them, regardless of size, is there evidence of stelar cambial activity.

The greater portion of the root is made up of cortex (fig. 11). Despite the fact of its being composed of relatively thin-walled cells and its lacunar nature, the cortex is often found with almost complete preservation. In some instances it has remained intact while all traces of the stelar tissues have disappeared. The lacunae are disposed with striking regularity from the stele to the outer region of the cortex (figs. 11, 13, 14, 15).

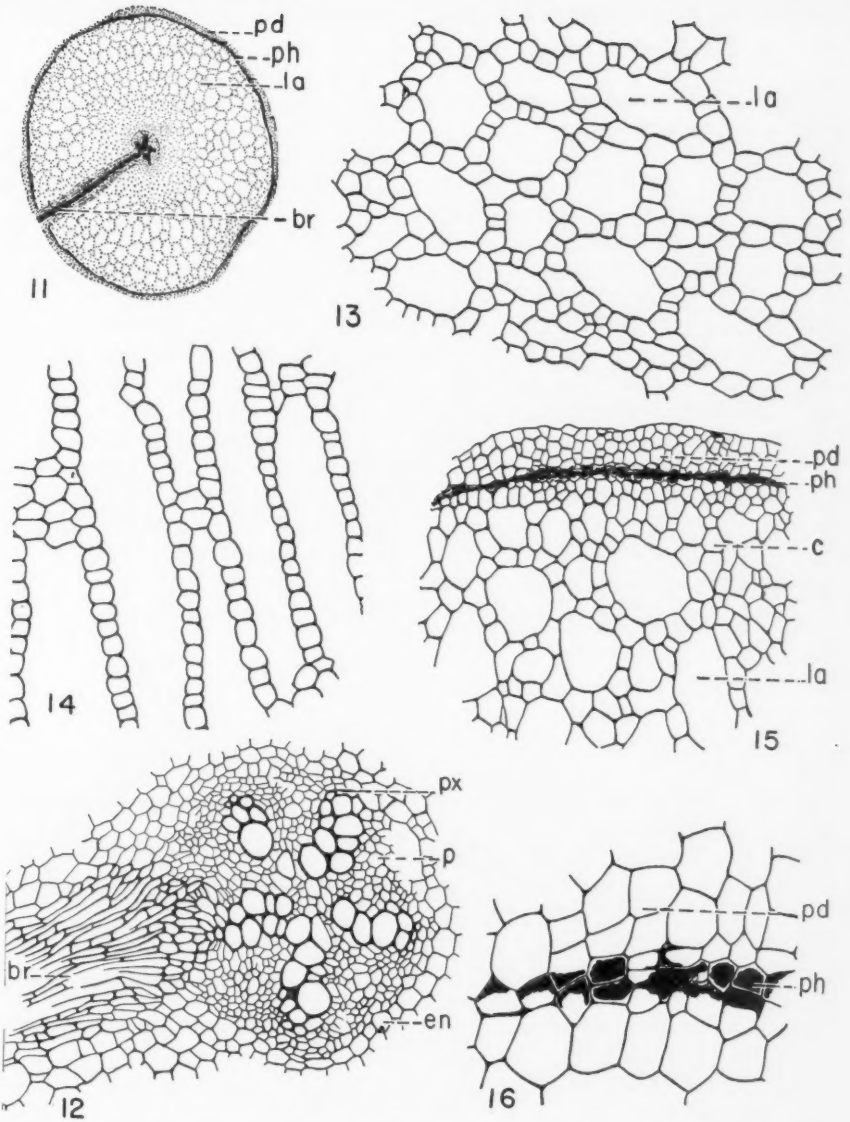
At the peripheral region of the rootlet there is a zone which has been described as "a narrow zone of heavy-walled fibrous cells, two or three cells in width" (2). Specimens of rootlets in which the preservation is more nearly complete and the limits of the cells discernible demonstrate this tissue to be phellogen (figs. 11, 15, 16). The cells of the phellogen and its immediate derivatives have some dark contents which give them the illusion of being thick-walled and which render the zone opaque under low magnification (figs. 11, 15).

The outermost zone of the cortex is composed of four or five layers of cells, many of which suggest that they may be of secondary origin; therefore, the tissue is thought to be periderm (figs. 15, 16, *pd*).

Branching of *Psaronius* roots seems exceedingly sparse. Thus far the writer has found no mention of lateral roots in the literature; furthermore, it was after an examination of many different roots as well as many serial sections that the branch root of figures 11 and 12 was discovered.

DISCUSSION.—The young rootlets of figure 7 are not assignable to any known species nor do they furnish a clue to the origin and nature of the controversial interstitial tissue. Nevertheless, they do represent a stage in developmental anatomy within the genus *Psaronius* and are here recorded that they may serve as a basis of departure in further investigations of the genus.

The anatomical differences between the cortex of the young rootlets (figs. 7–9), in which there is a narrow zone of thin-walled inner cortex surrounded by a zone of sclerotic cells, and that of the mature roots (fig. 11), where the cortex is thin-walled and lacunar, seemed difficult to reconcile until the peripheral phellogen was discovered. Since there is a phellogen, it would appear that the lacunar tissue is secondary tissue derived from the phellogen, which in turn had its



FIGS. 11–16.—*Psaronius* rootlets. Fig. 11, diagram of transection of mature root with lateral root: *pd*, periderm; *ph*, phellogen; *la*, cortical lacuna; *br*, longisection of stelar region of branch root. Fig. 12, detail of stele of root of fig. 11, showing origin of branch root: *en*, endodermis. Fig. 13, detail of portion of transection of lacunar region of cortex. Fig. 14, longisection through lacunar region of cortex. Fig. 15, transection through peripheral region of cortex showing position of phellogen. Fig. 16, detail of phellogen and its derivatives.

origin in the inner cortex of the young roots. Such a derivation would make this lacunar cortex comparable in its origin and appearance to aerenchyma of some of the extant aquatics.

Summary

1. Some isolated vegetative fragments of two groups of Carboniferous plants, *Sphenophyllum* and *Psaronius*, are described. The fragments are from different sources, from Indiana, Illinois, and Texas, but all are from coal fields which were said to be part of the Allegheny formation of the Pennsylvanian system.

2. The *Sphenophyllum* fragments consist of stem tips, their associated leaves, and small portions of two mature stems. One of the mature stems is noteworthy for its exceptional size, measuring 1.5 cm. in diameter, which makes it one of the largest, if not the largest, on record. This stem also possesses irregularities of growth simulating growth rings.

3. A review of the anatomical features of the different regions of *Sphenophyllum* leads to the belief that the plant was of a trailing or scrambling (lianoid) habit.

4. Adventitious rootlets from two regions of *Psaronius* are described: (*a*) young rootlets from the upper region of the stem, where they are crowded and held together by interstitial tissue, have primary tissues only, and are incompletely lignified; and (*b*) from the lower region where the rootlets are mature and free from one another.

5. In the mature rootlets there is demonstrated a peripheral phellogen from which the lacunar cortex appears to have been derived. There is also demonstrated an instance of branching.

Mount Holyoke College
South Hadley, Massachusetts

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STIMULATION BY WIND MOTION OF COLLENCHYMA FORMATION IN CELERY PETIOLES

FRANK D. VENNING

Introduction

The younger, softer portions of plants, such as succulent stem tips and leaf petioles, have often been observed to contain a relatively large proportion of collenchymatous tissue, usually strategically placed to give stiffness or support to the young tissues at points where they may be subjected to stress (2). As these tissues mature and as adequate xylem is differentiated for support, the collenchyma is often absorbed. Such appearances of collenchymatous tissue suggest that this tissue might be produced as a response to a physiological stimulus, such as wind motion, rather than as a heritable part of the anatomy of a plant. This experiment was undertaken to determine what effect the stimulus of wind motion would have on the number and size of collenchyma bundles in the petioles of celery.

Material and methods

Two groups of celery seedlings about 6 inches tall were transplanted to greenhouse flats filled with ordinary potting soil. For one group, which served as the control, high glass sides were affixed to the flat to prevent wind currents from striking the plants, but the top was left partially open to permit the escape of water vapor.

The other group was grown in an open flat and subjected to continuous wind from a 16-inch Emerson electric fan running at low speed. The two groups were grown side by side in the same greenhouse, which insured that the temperature and light factors would be comparable. The soil was kept moist for both groups of plants at all times.

The first material was collected for study 27 days after transplanting, when a plant was removed from each series. Subsequent collections were made in 29, 32, 34, and 41 days, respectively, after transplanting. At 41 days the plants in the control were 14 inches tall and relatively slender; those growing in the wind were shorter, stalkier, and had a more spreading habit. The two outermost leaves of each plant were discarded, and from the next five leaves two freehand cross sections were made of the petiole, one just above the membranaceous petiolar wings, and the other one about 1 cm. above the termination of the wings.

The celery petiole has from seven to nine major vascular bundles extending parallel to its long axis. Each bundle has, immediately dorsal and, to a lesser extent, immediately ventral to it, a collenchymatous bundle sheath. On the dorsal side of the petiole is a series of ribs which usually correspond to the bundles in number; each rib contains a relatively large strand of collenchyma. As ESAU (1) has shown, it is this latter collenchyma bundle which causes the most "stringiness" in celery. The present study was restricted to these areas of collenchyma.

All the collenchyma bundles in each section were outlined at 100 diameters on a sheet of paper with the aid of a camera lucida, and the area of each bundle computed with a planimeter. As the collen-

chyma bundles are of fairly regular outline, quite accurate relative areas may be obtained by this method. A total of 782 bundles from fifty sections of twenty-five petioles from five plants in each series was studied; measurements were obtained for the relative amount of collenchyma in the two series of plants.

Observations

The plants grown in the wind were subjected to two environmental factors for which values differed from those in the control: (*a*) a considerable amount of mechanical motion of the leaves, and (*b*) the xerophytic effect of the wind, with consequent increase of transpiration rate. Both of these factors must be considered together as having brought about differences between the two series. In nature, however, land plants are seldom subjected to motion without wind, so these two factors probably should not be considered separately in an experiment of this kind.

As mentioned, the habit of the two groups became noticeably different after the first week, and the difference in habit increased as the experiment progressed. The control plants were taller, with more slender petioles, and their stalks were more flexible to the touch.

The series of plants growing in wind showed only 2.2% more collenchyma bundles per petiole than did the control. This number cannot be considered as an appreciable difference, since it comes within the limits of the margin of probable error. This series had collenchyma bundles 45% larger than those of the control and their petioles contained a 50% greater area of collenchyma than did those of the control plants. Table 1 lists the average relative measurements obtained in the two series and the average totals for all five plants in each series.

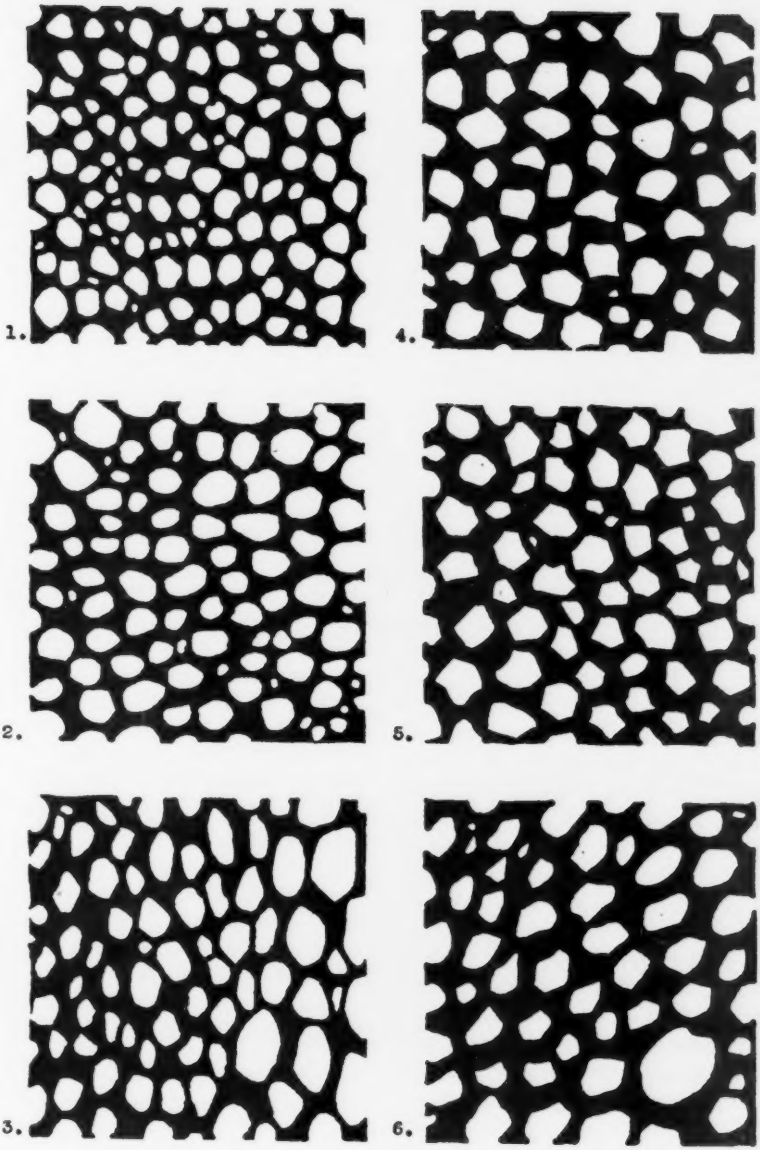
The collenchyma differentiated in the plants growing in the wind had thicker walls than that in the motionless plants (figs. 1–6). There were more cells per unit area of collenchyma in the motionless plants. The collenchyma bundles opposite the midrib showed the greatest difference, with twice as many cells per unit area.

It may be concluded that wind motion has no appreciable effect on the anatomical pattern in celery petioles, i.e., the number and kind of structures differentiated, and their arrangement to one another. It has, however, a marked effect on the degree to which the collenchyma bundles are expressed.

TABLE 1

AVERAGE RELATIVE COLLENCHYMA MEASUREMENTS

	Control plants	Wind plants
Plant 1:		
Area of collenchyma per petiole	82.64	142.36
No. of bundles per petiole	7.4	8.3
Average area per bundle	11.17	17.15
Plant 2:		
Area of collenchyma per petiole	104.9	115.6
No. of bundles per petiole	6.9	7.5
Average area per bundle	15.20	15.42
Plant 3:		
Area of collenchyma per petiole	89.52	160.59
No. of bundles per petiole	7.7	8.0
Average area per bundle	11.63	20.07
Plant 4:		
Area of collenchyma per petiole	129.68	167.59
No. of bundles per petiole	8.0	7.7
Average area per bundle	16.21	21.76
Plant 5:		
Area of collenchyma per petiole	138.9	231.4
No. of bundles per petiole	8.4	8.3
Average area per bundle	16.5	27.9
Average totals for five plants from both series:		
Area of collenchyma per petiole	109	164
No. of bundles per petiole	7.7	7.9
Average area per bundle	14.5	20.46



FIGS. 1–6.—Typical cross sections of portions of collenchyma bundles from celery petioles grown in (figs. 1–3) a motionless environment or in (figs. 4–6) constant wind, showing relative cell size and wall thickness. Figs. 1, 4, midrib collenchyma bundles. Figs. 2, 5, first lateral collenchyma bundles. Figs. 3, 6, extreme lateral collenchyma bundles. ×300.

Summary

1. Comparisons were made of the size and number of collenchyma bundles differentiated in two series of celery seedlings grown in constant wind and in a windless environment.

2. Celery subjected to wind motion differentiates approximately the same number of collenchyma bundles per petiole as plants from a windless environment.

3. Wind motion stimulates the development of collenchyma within the bundles, resulting in (*a*) larger areas of collenchyma, (*b*) heavier thickenings of the cell wall, and (*c*) collenchyma cells of larger diameter.

This experiment was conducted while the author was in attendance at the State University of Iowa, and he is especially indebted to Dr. ROBERT L. HULBARY of the Department of Botany of that institution, both for providing the necessary experimental facilities and for his helpful suggestions during this study.

BOTANY DEPARTMENT
UNIVERSITY OF MIAMI
MIAMI, FLORIDA

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APPARENT ANTAGONISTIC EFFECTS OF GROWTH-REGULATORS

PAUL C. MARTH[1] AND JOHN W. MITCHELL[2]

Introduction

In studying the effects of growth-regulating substances on the growth and maturation of fruits, it was noted that hard green banana fruits treated with 2,4-dichlorophenoxyacetic acid (2,4-D) and stored in airtight rooms or containers failed to respond to the ripening effects of the 2,4-D. Comparable fruits treated and placed in well-ventilated rooms, on the other hand, ripened at a faster rate and more uniformly than did untreated ones (1). Gaseous substances that came from the fruits were apparently antagonistic to the ripening effects of the 2,4-D.

Ethylene is generated by ripening bananas and is sometimes applied artificially to hasten their ripening. It was therefore thought that the presence of ethylene might in part account for the effect, and experiments with various concentrations and combinations of pure ethylene and 2,4-D were conducted. The addition of 2,4-D greatly reduced the ripening effects of the ethylene. Thus, a growth-regulating substance of known composition (2,4-D) had an antagonistic effect on the results produced by action of another growth-regulator of known composition (ethylene).

Methods

For the experiments, hard green bananas, grading 3/4 full or less, were obtained as they were unloaded following shipment from Central America.[3] Two to

[1] Horticulturist, [2] Physiologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Md.

[3] Fruit was furnished by Fruit Dispatch Co., New York City; 2,4-D ester, by Baker Chemical Co., Phillipsburg., N.J.; and Tween 20, by the Atlas Powder Co., Wilmington, Del.

three stems of fruits having ten to fifteen hands per stem were used in each experiment. The hands were separated from the stem and distributed at random in groups of equal numbers so that each lot or treatment consisted of four to six hands containing forty to ninety individual fruits.

The rate of fruit-color change was estimated by means of a standard color chart published by the Fruit Dispatch Company. Grades 1, 2, 3, and 4 designate colors from green to greenish-yellow, 5 full yellow with green tips, 6 full yellow, and 7 fully yellow with light speckles. The rate of softening was measured by means of a fruit pressure-tester; eight fruits were used per test, and two readings were made on the flesh of each fruit after both ends had been removed for a distance of about 1 inch. The pressure required to force a rounded plunger $\frac{7}{16}$ inch in diameter into the fruit to a depth of $\frac{5}{16}$ inch was recorded in pounds and the average computed of the sixteen readings for each treatment.

Experimentation and results

To study the effect of gaseous substances from ripening fruits on the ripening effect of 2,4-D, fifteen hands of hard green bananas were separated at random into three lots. The first lot was left untreated and kept well ventilated in open air. The second lot was dipped in an aqueous mixture containing 1000 p.p.m. of 2,4-D and 1% Tween 20, then kept well ventilated in the open air. The third lot was treated with the 2,4-D in a similar manner, surface-dried, and immediately sealed in a 40-gallon metal can. The can was left sealed for 5 days, during which time the temperature varied from 62° to 77° F.

Of the treated fruits, ventilated ones softened and became yellow (pressure test 4.3 lb., color grade 4) during this 5-day period. In contrast, the inclosed ones remained hard (12.8 lb.) and deep green in color (grade 2). Untreated ventilated fruits remained hard and green.

This experiment was repeated with fruits held at a temperature of 68°–72° F. The sealed lot was left inclosed for 5 days, then exposed to open air. Nine days after treatment (5 days sealed, 4 days exposed) the treated fruits that had been sealed were still green (color grade 3), hard (average 12.8 lb.), and unpalatable, while comparable treated and ventilated ones had developed full yellow color (color grade 6) and were soft (5.8 lb.) and sweet. During this period the ventilated untreated ones showed various color grades (2–7) and degrees of softness (from 4 to 28, with an average of 21.8 lb.).

An experiment was designed to determine the approximate amount of ventilation required to offset the antagonistic effect of gaseous substances from the fruits on the ripening potency of 2,4-D. Separate sealed containers of 2,4-D-treated fruits were ventilated during a 5-day period by removing the lids and completely changing the air in the container once daily, twice daily, and every other day. Ripening of these fruits was compared with that of others treated and stored in a well-ventilated room. One treated lot was left sealed continuously as a check.

There was no significant difference in the rate of ripening of treated fruits ventilated twice daily and of treated ones ventilated continuously (table 1). Ventilation once a day or every other day failed to lower the concentration of gaseous substances from the fruits to the point where they were no longer antagonistic to the 2,4-D effect. Fruits that were treated and sealed continuously

ripened to a slight extent (15.4 lb. average pressure test), but these were still hard, green in color, and unpalatable after the 5-day period.

In all these experiments fruits that were treated with 2,4-D and then inclosed failed to respond in an appreciable degree to the ripening effects of the 2,4-dichlorophenoxyacetic acid, a nonvolatile form of the chemical. Other tomato plants were inclosed with untreated fruits as a check. In every instance the plants developed strong epinastic responses and some stem curvature when sealed with either the treated or the untreated fruits. Apparently some volatile growth-regulating substance, such as ethylene, was given off from both the treated and the untreated fruits as they ripened.

TABLE 1

EFFECT OF VENTILATION ON RIPENING OF 2,4-D-TREATED BANANAS. FIGURES REPRESENT DEGREE OF FIRMNESS BASED ON PRESSURE TESTS AND EXPRESSED IN POUNDS. OBSERVATIONS MADE AFTER 5 DAYS' TREATMENT

UNTREATED	2,4-D-TREATED*				
Ventilated continuously	Ventilated continuously	Ventilated twice daily	Ventilated once daily	Ventilated every other day	Sealed continuously
23	6	8	7	7	16
24	6	7	9	8	16
21	6	6	9	7	16
22	6	6	8	7	17
16	6	5	8	8	14
17	6	5	8	9	13
19	5	6	7	9	16
18	6	6	7	8	15
21	6	6	9	11	15
23	6	6	8	10	14
23	5	7	9	13	17
23	6	6	9	13	17
23	6	6	8	13	16
24	6	6	8	10	16
23	6	6	8	9	14
25	6	6	8	13	14
21.6†	*5.9†*	*6.1†*	*8.1†*	*9.7†*	*15.4†*

* Aqueous solution dip, 2,4-D (acid) 1000 p.p.m. with 1.0% of Tween 20.

† Figures in italics are averages.

2,4-D. There were apparently gaseous substances given off by the fruits that were antagonistic to the ripening effects of 2,4-D.

Attention was next directed toward the effect of combinations of 2,4-D and ethylene on the rate of ripening of bananas. A preliminary test was first made to determine whether the 2,4-D treatment affected the natural evolution of ethylene by the fruit. Young, rapidly growing tomato plants were inclosed in gastight cellophane cases (volume 1 cu. ft.) along with bananas treated with

A series of experiments was next made to determine whether artificially applied ethylene might counteract the 2,4-D ripening effect on bananas. In such an experiment one lot was left untreated and designated as control. A second lot was dipped in an aqueous mixture containing 500 p.p.m. of the amyl ester of 2,4-D and 1.0% Tween 20. A third lot was sealed in a 40-gallon can containing ethylene at the rate of 1 part per thousand of air (the recommended commercial dosage). These fruits remained sealed for 1 hour, after which time they were removed from the can and dipped in the solution of 2,4-D. All fruits were then hung up to ripen in a well-ventilated room in which the air temperature ranged from 72° to 78° F. At intervals of 1 to 2 days during the following week the softness of the fruits was measured.

Untreated fruits remained hard and green in color. Those treated with ethylene alone softened within a period of 3 days. Fruits treated with 2,4-D alone or with combinations of ethylene and 2,4-D were only partially ripe at the end of a 3-day period. The average pressure test values in pounds at the end of this period were: untreated fruits, 23.7; ethylene plus 2,4-D, 10.3; and 2,4-D alone, 9.1. No evidence was obtained to indicate that ethylene reduced the effectiveness of 2,4-D in ripening the fruit.

In a repetition of this experiment with the same methods, similar results were obtained. In this test no significant difference was found between the rate of ripening of fruit treated with 2,4-D alone and that of those treated with the combination of 2,4-D and ethylene. Nine days after treatment untreated fruits tested an average of 22.9 pounds; ethylene plus 2,4-D, 8.4 pounds; and those treated with 2,4-D alone, 7.4 pounds.

In still another experiment fruits treated with the combination of 2,4-D and ethylene ripened slightly more than did others treated with 2,4-D alone. After 3 days at a temperature varying from 72° to 80° F. the average pressure test of 2,4-D-treated fruits was 8.1 pounds, while that of fruits treated with a combination of 2,4-D and ethylene was 5.9. Untreated fruits remained hard and green (pressure test 22.8) during this period. In all three experiments there was no indication that ethylene reduced the effectiveness of 2,4-D in ripening bananas.

Similar experiments were made to learn whether a very minute amount of the ethylene gas (1:100,000) would alter the ripening effect of 2,4-D. Fruits that received a combination treatment consisting of ethylene plus 1000 p.p.m. 2,4-D had an average pressure test of 14.4 pounds; those treated with 2,4-D alone, 14.6 pounds. During this 5-day period the untreated control remained hard and green (23.7 lb. average pressure). The small amount of ethylene used did not alter the ripening effect of the 2,4-D.

A similar experiment with an even smaller amount of the gas (3.4 parts per million of air) and 1000 p.p.m. of the 2,4-D gave similar results. Thus ethylene added in a wide range of concentrations did not alter the ripening effect of the acid.

A series of experiments was made to compare the effects of ethylene with the effects of a combination of ethylene and 2,4-D on the rate of ripening. In each experiment ethylene greatly accelerated ripening, but the presence of 2,4-D inhibited this ripening effect of the ethylene (table 2).

An additional experiment demonstrated that 2,4-D was also antagonistic to the ripening effects of naturally occurring ethylene. Five hands of bananas, selected at random, were dipped in an aqueous solution containing 1000 p.p.m. of nonvolatile 2,4-dichlorophenoxyacetic acid and 1% Tween 20. These fruits were then sealed in a can together with an equal number of untreated ones. Five other hands selected at random were left untreated in open air as checks.

The can was opened after 5 days and pressure tests were made. Both the untreated fruits in open air and the inclosed 2,4-D-treated fruits remained hard and green during this period (17.4 and 15.7 lb. average pressure test, respectively), while untreated fruits sealed together with treated ones ripened (8.6 lb. average pressure test). This experi-

TABLE 2

EFFECT OF ETHYLENE AND OF 2,4-D IN COMBINATION WITH ETHYLENE ON SOFTENING OF BANANAS, IN COMPARISON WITH SOFTENING OF UNTREATED FRUITS. FIGURES REPRESENT AVERAGE OF SIXTEEN PRESSURE TESTS EXPRESSED IN POUNDS

Experiment	Days after treatment	Untreated	Ethylene	2,4-D+ ethylene*
I	4	23.7	5.2	10.3
II	3	22.8	9.3	15.8
III	3	22.8	8.7	12.3

* 2,4-D used at rate of 1000 p.p.m. in 1.0% aqueous solution of Tween 20; ethylene, 1:1000 of air in experiment I, and 1:10,000 in experiments II and III.

ment was repeated, and results similar to those described were obtained. Thus untreated fruits ripened more quickly when inclosed in an airtight container, probably owing to the effect of naturally produced ethylene that accumulated in the confined atmosphere. In contrast, fruits treated with the acid failed to respond to the same atmosphere that stimulated ripening of untreated ones. It is, therefore, concluded that 2,4-D was antagonistic to the ripening effects of ethylene produced naturally, as well as to that which was artificially applied. It has been reported (**2**) that growth-regulators, such as naphthaleneacetic acid and others, inhibit the storage scald of apple fruits, a physiological disorder thought to be due to the accumulation of gaseous substances given off by the fruits as they ripen.

Summary

1. The effect of (*a*) 2,4-dichlorophenoxyacetic acid (2,4-D), (*b*) ethylene, (*c*) combinations of 2,4-D and ethylene, and (*d*) gaseous substances from ripening bananas on the rate of ripening of bananas was studied.

2. Some volatile substance, or substances, was given off by ripening bananas that was antagonistic to the ripening effects of 2,4-D on bananas.

3. Synthetically prepared ethylene apparently did not reduce the ripening effects of 2,4-D on bananas.

4. On the contrary, 2,4-D greatly reduced the effectiveness of either chemically prepared or naturally produced ethylene in accelerating ripening of bananas.

5. Application of a nonvolatile form of 2,4-D to bananas did not prevent the evolution of gaseous substances from the fruit which were capable of inducing epinastic responses in tomato plants. The chemical nature of these substances was not determined.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

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PITTING IN THE CELL WALLS OF THE SCUTELLUM OF ZEA MAYS[1]

BARBARA L. WILCOX

No previous mention of the scutellar pits in corn observed in this laboratory has been found in the literature, either in specific discussions of the scutellum (5) or in more comprehensive descriptions of the pistillate spikelet and the developing caryopsis (2, 4). The corn germ employed in this study was an ordinary yellow dent variety of the type used in the American wet-milling industry. Its exact origin or variety was unknown, since it was obtained after it had been shelled. Nevertheless, the particular structure to be discussed can, I believe, be considered as common to *Zea mays* because it was observed in a large number of kernels over a 5-month period from April to September, 1948, in several kernels of a sweet seed corn, and also in several kernels of a large Argentine variety.

The accompanying photomicrograph (fig. 1) shows the rather large and prevalent simple pits which occur in the cell walls of the scutellum. Their demonstration is relatively easy, and indeed they can be observed in the unstained condition, although the section photographed was stained with 0.1 *N* iodine in KI and washed with distilled water in order to enhance contrast in the negative. Longitudinal sections, 25–50 μ in thickness, were cut parallel to the face of the germ, using a small hand microtome with a freezing attachment. Some sections were stained with iodine, some with methylene blue, and some with ruthenium red. The pit membranes, which extend to form the intercellular substances, stained violet with the methylene blue and red with the ruthenium dye, indicating the presence of pectic compounds.

An attempt was made to demonstrate the presence of plasmodesmata across these pit membranes, using a modification of the technique described by PRATT and DUFRENOY (3). Fresh germs were separated from the endosperm, cut transversely into small pieces, and soaked in a 0.5% solution of 2,3,5-triphenyltetrazolium chloride for about 30 minutes or until they were deep pink. Sections were then cut from these pieces with the apparatus mentioned above. Presumably, the protoplasmic connections, if present, would have appeared red owing to the reduction of the colorless tetrazolium salt and its prompt deposition as a red formazan (1). Occasional slides gave strong indications of these pits in

FIG. 1. Cell contents have been brushed away, exposing very prevalent simple pits. × 440.

[1] Contribution from the George M. Moffett Research Laboratories of the Corn Products Refining Company.

side views down on cut walls, and in one instance the surface of a pit seemed to show small red dots. The demonstration of such connections was neither easy nor consistent, however, and, while their presence seems plausible, it is hoped that further confirmation can be obtained to clarify this point.

2110 KAKELA PLACE
HONOLULU, T.H.

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CURRENT LITERATURE

The Use of Isotopes in Biology and Medicine: A Symposium. Madison: University of Wisconsin Press, 1948. Pp. xiv+445. Illus.

This book consists of twenty essays which were presented as a symposium at the University of Wisconsin during the week of September 10, 1947. The quality of the contributions is exceptionally high, and the topics covered are well chosen and arranged.

The book opens with a brief account of the history of isotopes in biochemistry (HANS T. CLARKE). This is followed by three papers on ways of obtaining isotopes: separation of stable isotopes (HAROLD C. UREY); preparation of radioactive isotopes (GLENN T. SEABORG); and availability of both stable and radioactive isotopes from the Atomic Energy Commission (PAUL C. AEBERSOLD). Next follow three papers on the assay of isotopes: the detection of stable isotopes, with special reference to the mass spectrometer (ALFRED O. NIER); principles of measurement of radioactivity (CHARLES D. CORYELL); and the application of these principles to the assay of radioisotopes in biological material (MARTIN D. KAMEN). These papers are followed by a short review of procedures used in incorporating tracer atoms into organic molecules (DONALD B. MELVILLE).

After the foregoing articles on techniques, there follow six reviews of results obtained with tracers: in protein metabolism (DAVID B. SPRINSON); in intermediary carbohydrate metabolism (HARLAND G. WOOD); in intermediary metabolism of lipids (KONRAD BLOCH); in mineral metabolism (DAVID M. GREENBERG); in iodine metabolism and thyroid function (I. L. CHAIKOFF and A. TAUROG); and in general medicine (JOSEPH G. HAMILTON).

Certain therapeutic applications of radiophosphorus are described by BYRON E. HALL and of radioactive iodine by SAUL HERTZ. Health hazards in the use of radioisotopes are discussed by WILLIAM F. BALE from the standpoint of the physicist and by JAMES J. NICKSON from the standpoint of the physician.

The last two papers are thought-provoking essays by HAROLD C. UREY on international aspects of atomic energy and by FARRINGTON DANIELS on the development and applications of atomic energy.

The book is clearly written. Although lithoprinted, it is quite legible. The relatively few errors are concisely listed on a single sheet. The book is illustrated with photographs of the contributors and of the chairmen of the various sessions.

The authoritative nature of most of the contributions to this book make it a most useful addition to the library of the experimental biologist who is interested in tracers.—RAYMOND E. ZIRKLE, *University of Chicago.*

The Study of Plant Communities. By HENRY J. OOSTING. San Francisco: W. H. Freeman & Co., 1948. Pp. 389+190 figs.+11 tables. $4.50.

This book is intended as an introductory textbook in plant ecology. The plant community is made the basis of the book because of the desire of the author to make the subject matter as stimulating as possible. Another reason for this approach, that plants do not live alone but together in communities, is implied but not stated. The material is carefully selected, is presented in a clear, terse fashion, and is well illustrated. As seems proper in the preparation of a college textbook in plant ecology, the author assumes a background of botanical and scientific experience, usually not attained before the junior year, for adequate appreciation of the course.

The plan and outline of the book are very simple, and yet the student is at least introduced to most of the subject matter of plant ecology. There are only five parts, including a ten-page Introduction. The presentation proceeds from this brief but adequate introduction to the second part, a discussion of "the plant community." Naturally some difficulty is encountered in describing the general nature of the plant community in specific terms before the subject of the environmental factors of the community is presented. This description is followed by an interesting, well-illustrated, and documented presentation of methods and results of quantitative and qualitative vegetational analysis of the plant community. More emphasis could be placed on methods such as the weight-list and weight-estimate quadrats in evaluating and comparing plant communities. The third part deals with the analysis of the environment and its relationships to the community. Although the factors are classified as climatic, physiographic, and biological rather than on the basis of factor-plant interactions, this latter concept, as well as that of the interrelation of plants living under an environment of interacting factors, is well developed in terms of cause and effect.

Emphasis on the effects of the community on its environment lays the foundation for the presentation of plant succession and climax communities in the fourth part of the book under the title "Community Dynamics." The subject of plant succession is concisely and yet lucidly developed. Almost one-fifth of the book is given over to the regional distribution of native plant communities in North America before the advent of the white man under the title "Present Distribution of Climaxes." Some few

examples are given of other than climax natural communities, but the many communities modified by cropping and other cultural practices receive only general treatment in the chapter on biological factors. The short chapter on the dynamic nature of climax communities presents a concept, often omitted, which seems to be essential in even an introductory course in ecology. Practical considerations of the subject are discussed separately in Part V. The author represents the problem of the prudent and skilful use of his natural resources by man as fundamentally ecological. The same principles apply, in determining the degree of control which should be exerted and in effecting this control, in the practical fields of plant and animal production and in the general utilization of resources, as apply in natural communities of organisms.

The selected bibliography of two hundred and seventy-six titles tends to give the student an erroneous idea that the research contributions in ecology have been made by a relatively small number of workers.

The results of two trial periods indicate that the book is usable as a text in an introductory course in plant ecology. The course may be enriched and supplemented by the use of available texts and source books in ecology and by a more nearly complete bibliography.—JOHN M. AIKMAN.